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CONTENS

EDITORIAL ARTICLE

- Jakub Adamczyk, Dominik Sieroń, Karolina Sieroń, Aleksander Sieroń, Ewa Kucharska
METHODS OF SPECTRAL DIAGNOSTICS IN MODERN PREVENTION OF ONCOLOGICAL DISEASES 1313

ORIGINAL ARTICLES

- Galyna A. Yeroshenko, Larysa Ya. Fedoniuk, Konstantyn V. Shevchenko, Denys R. Kramarenko, Anastasiia I. Yachmin, Olena V. Vilkhova, Tetiana A. Skotarenko
STRUCTURAL REORGANIZATION OF THE RATS' SUBMANDIBULAR GLANDS ACINI AFTER THE INFLUENCE OF 1% METHACRYLATE 1318
- Jarosław Świrta, Michał Romaniszyn, Marcin Barczyński
RISK FACTORS OF MALIGNANCY IN PATIENTS WITH FINE NEEDLE ASPIRATION BIOPSY RESULTS INTERPRETED AS "SUSPICIOUS FOR FOLLICULAR NEOPLASM" 1323
- Ivanna I. Gorban, Arsen A. Hudyma, Roman V. Maksymiv, Iryna V. Antonyshyn
INFLUENCE OF TWO-HOUR TOURNIQUETS ISCHEMIA OF LIMB AND ACUTE BLOOD LOSS ON SYSTEMIC DISORDERS OF THE BODY IN THE REPERFUSION PERIOD (EXPERIMENTAL STUDY) 1330
- Svitlana I. Zhuk, Oksana D. Shchurevska
MATERNAL PSYCHOSOCIAL STRESS AND LABOR DYSTOCIA 1334
- Martyna Rożek, Zuzanna Śmiech, Marcin Kolacz, Dariusz Kosson
PAIN RELIEF DURING LABOR – WHAT DO WE KNOW AND WHAT SHOULD WE KNOW? RESEARCH BASED ON THE STUDENT POPULATION IN WARSAW 1339
- Svetlana A. Pavlenko, Elena V. Pavlenkova, Irina M. Tkachenko, Alla I. Sidorova, Dmytro M. Korol
PROGNOSTIC INDICES OF PATHOLOGICAL TOOTH WEAR DEVELOPMENT 1345
- Igor V. Yaishen, Karina Y. Andrienko, Irina O. Pereshivaylova, Leah G. Salia, Elena O. Berezhna
EVALUATION OF PATIENT'S QUALITY LIFE WITH JOINT AND MUSCLE DYSFUNCTION 1350
- Oleksander V. Kovtunencko, Bohdan V. Shportko, Serhey M. Tymchuk
ANALYSIS OF CYTOKERATINE 19 EXPRESSION LEVEL BY IMMUNOCYTOCHEMICAL DIAGNOSTIC METHOD IN LARYNGEAL CANCER PATIENTS WITH REGIONAL METASTASES 1355
- Tetiana V. Budnik, Tetiana B. Bevzenko
A TEN-YEAR ANALYSIS OF CHANGES IN THE SENSITIVITY OF THE LEADING UROPATHOGEN TO ANTIBACTERIAL AGENTS IN CHILDREN WITH URINARY TRACT INFECTION IN THE NEPHROLOGY DEPARTMENT 1360
- Aleksey A. Oparin, Iryna O. Balaklytska, Olga G. Morozova, Anatolii G. Oparin, Ludmila O. Khomenko
MECHANISMS OF INSOMNIA FORMATION WITH GASTROESOPHAGEAL REFLUX DISEASE, TAKING INTO ACCOUNT THE PSYCHOSOMATIC STATUS IN YOUNG PEOPLE 1365
- Volodymyr V. Kasian, Volodymyr D. Sheiko, Tetiana V. Mamontova, Liudmyla E. Vesnina, Oksana A. Shlykova
PROCALCITONIN IN EARLY PREDICTION OF ACUTE SEVERE PANCREATITIS 1370
- Olga M. Shvets, Olga S. Shevchenko, Liliia D. Todoriko, Rostyslav S. Shevchenko, Volodumyr V. Yakimets, Oleksandra I. Choporova, Ganna L. Stepanenko
CARBOHYDRATE AND LIPID METABOLIC PROFILES OF TUBERCULOSIS PATIENTS WITH BILATERAL PULMONARY LESIONS AND MYCOBACTERIA EXCRETION 1373
- Olexandra V. Tiazhka, Zoriana V. Selska
APPLICATION OF VITAMIN D IN DIFFERENT DOSAGE TO TREAT CHILDREN WITH ALLERGIC DISEASES 1377
- Agnieszka Wiorek, Milena Horodecka, Lukasz J. Krzych
CLINICAL USEFULNESS OF THE INTERNATIONAL RENAL RESEARCH INSTITUTE OF VICENZA (IRRI) SCORE IN THE INTENSIVE CARE SUBJECTS WITH RENAL FAILURE: SINGLE-CENTRE EXPERIENCE 1384
- Olena F. Mruh
ASSESSMENT OF THE ADAPTIVE PERSONAL POTENTIAL OF THE PATIENTS WITH PARANOID SCHIZOPHRENIA 1391
- Oleksandra Ya. Pryshliak, Tetiana O. Nikiforova, Zoriana R. Tylishchak, Oleksandr P. Boichuk, Nadiya V. Vaskul, Oksana V. Kopchak, Andriy L. Protsyk
RETROSPECTIVE ANALYSIS OF LEPTOSPIROSIS MORBIDITY IN IVANO-FRANKIVSK REGION (EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS) 1397
- Sergiy M. Pyvovar, Iurii S. Rudyk, Mykola P. Kopytsya, Tetiana V. Lozyk, Valentina Ir. Galchinskaya, Tetiana O. Chenchik
THE EFFECT OF BETA-BLOCKERS ON A COURSE OF CHRONIC HEART FAILURE IN PATIENTS WITH A LOW TRIIODOTHYRONINE SYNDROME 1402
- Tetiana V. Ivakhniuk, Viktoriia M. Holubnycha, Vladyslav A. Smiiianov, Lesia A. Rudenko, Yevgen V. Smiiianov
ANTIBIOTIC RESISTANCE OF THE NASOPHARYNX MICROBIOTA IN PATIENTS WITH INFLAMMATORY PROCESSES 1415
- Mykhailo S. Myroshnychenko, Olena O. Dyadyk, Victor D. Urzhumov, Nataliia V. Kapustnyk, Iryna V. Borzenkova, Larisa I. Selivanova, Inna I. Torianyk, Yuliia Ya. Fedulenkova, Iuliia M. Kalashnyk, Pavel V. Tkachenko, Varvara R. Hryhorenko, Dmytro V. Molodan, Serhii S. Myroshnychenko
CLINICAL AND MORPHOLOGICAL FEATURES OF THE OVARIAN BRENNER TUMOUR: CURRENT STATE OF THE PROBLEM 1420
- Oskar Kublin, Mariusz Stepień
EFFECT OF USING THE NIGHTSCOUT SYSTEM ON METABOLIC CONTROL, SAFETY, AND INCIDENCE OF COMPLICATIONS IN PATIENTS WITH DIABETES MELLITUS 1427
- Iryna M. Nikitina, Svetlana A. Smiyan, Kateryna O. Kondratiuk, Natalia V. Kalashnik, Anzhelika A. Sheve
CONDITIONS OF MICROELEMENTS EXCHANGE PROCESSES IN WOMEN'S PLACENTS IN INTRAUTERINE INFECTION OF THE FETUS 1434
- Grygoriy P. Griban, Natalia A. Lyakhova, Oleksii V. Tymoshenko, Zhanna G. Domina, Nadya Yu. Dovgan, Mykola Z. Kruk, Ivan V. Mychka, Pavlo P. Tkachenko, Bogdan S. Semeniv, Ganna P. Grokhova, Natalya O. Zelenenko, Kostiantyn V. Prontenko
CURRENT STATE OF STUDENTS' HEALTH AND ITS IMPROVEMENT IN THE PROCESS OF PHYSICAL EDUCATION 1438
- Kamila Wachuła, Joanna Furman, Beata Łabuz-Roszak
THE MEDITERRANEAN DIET IN THE PREVENTION OF CARDIOVASCULAR AND NERVOUS SYSTEM DISEASES – THE ASSESSMENT OF NUTRITIONAL KNOWLEDGE 1448
- Vladyslav V. Lyubchak, Viktoriia M. Plaksa, Ihor M. Pelo, Michael P. Kovalishyn, Viktor P. Lyubchak, Vladimir V. Horokh, Tetyana Iu Lisovenko, Viktoriia V. Ilyina, Liliya M. Khomenko
THERAPEUTIC PLASMAPHERESIS IN A COMPLEX TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS 1454

Tatyana O. Yastrub, Sergii T. Omelchuk, Andrii M. Yastrub DERMAL ABSORPTION OF DIQUAT AND POTENTIAL OCCUPATIONAL RISK	1459
Yuriy V. Gavrilenko, Andriy A. Laiko, Oleg F. Melnikov, Lilia M. Rudenko, Tatiana M. Osadcha CLINICAL AND LABORATORY PECULIARITIES OF CHILDREN'S STATE WITH CHRONIC TONSILLITIS ON TYPE 1 DIABETES	1465
Volodymyr V. Martsiniv, Oleg A. Loskutov, Andriy M. Stokan, Mihaylo V. Bondar EFFICACY OF PECTORAL NERVE BLOCK TYPE II VERSUS THORACIC PARAVERTEBRAL BLOCK FOR ANALGESIA IN BREAST CANCER SURGERY	1470
Oleksandr O. Belov, Nataliya G. Pshuk AGE AND GENDER FEATURES OF DEPRESSIVE AND ANXIETY SYMPTOMATICS OF DEPRESSIVE DISORDERS	1476
Tetyana O. Pertseva, Lyudmyla I. Konopkina, Alina O. Babenko A NEW PERSPECTIVE ON BRONCHIAL ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE OVERLAP: POTENTIAL DIAGNOSTIC CRITERIA	1480
Svetlana V. Kalinichenko, Kristina V. Melentyeva, Hans Manee, Natalia V. Dubinina, Natalia V. Zvereva, Inna I. Toryanik, Natalia G. Popova, Oleksandr V. Pakhomov STUDY OF ANTI-VIRUS ACTIONS OF METABOLITES OF <i>LACTOBACTERIA</i>	1484
Anatoly A. Avramenko FREQUENCY OF IMPLEMENTATION AND IMPACT OF APPENDECTOMY AT ACTIVATION OF THE PATHOLOGICAL PROCESS IN PATIENTS WITH CHRONIC NON-ATROPHIC GASTRITIS	1489
Iryna B. Romash, Vasyl G. Mishchuk THE FREQUENCY OF VISCERAL AND PHENOTYPIC MARKERS IN PATIENTS WITH THE COMBINATION OF UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE AND GASTROESOPHAGEAL REFLUX DISEASE	1492
Oleksandr A. Udod, Hanna S. Voronina, Olena Yu. Ivchenkova APPLICATION OF NEURAL NETWORK TECHNOLOGIES IN THE DENTAL CARIES FORECAST	1499
Lyudmyla V. Pakharenko, Volodymyr D. Vorobii, Nataliia Ya. Kurtash, Olena M. Kusa, Inna V. Kravchuk, Viktor M. Zhurakivskyi ASSESSMENT OF ESTROGEN RECEPTOR GENE POLYMORPHISM (T-397C VARIANT) IN PATIENTS WITH PREMENSTRUAL SYNDROME	1505
Oleg V. Kravets, Igor P. Fedzhaga, Vladimir I. Pivtorak, Oleg P. Fedzhaga, Iryna V. Bulko PERFORMANCE STATUS AND QUALITY OF LIFE AFTER RECONSTRUCTIONS OF BUCCAL MUCOSAL AND RETROMOLAR TRIGONE DEFECTS BY SKIN AND FASCIAL FLAPS IN ONCOLOGICAL PATIENTS	1510
Oksana V. Tymoshchuk, Oksana Y. Zhurakivska, Volodymyr V. Derpak, Iryna O. Kostitska, Iryna T. Tokar SPECIAL ASPECTS OF THE EFFICIENT DAILY ROUTINE OF STUDENTS AT MODERN EDUCATIONAL ESTABLISHMENTS OF DIFFERENT TYPES	1516

REVIEW ARTICLES

Farouq Ahmad Faleh Alazzam, Ali Jabbar Saleh, Khaled Khalaf Abed Rabbo Aldrou IMPACT OF PANDEMIC COVID-19 ON THE LEGAL REGULATION OF WORLD TRADE ACTIVITY USING THE EXAMPLE OF THE MEDICAL SUPPLIES	1521
Ewa Wielosz, Maria Majdan CLINICAL AND SEROLOGICAL PARAMETERS OF PROGRESSION AND PROGNOSIS IN PATIENTS WITH SYSTEMIC SCLEROSIS – A STATE OF THE ART REVIEW	1528
Sandra Kaija, Inga Kudeikina, Nataliya Gutorova MEDICAL AND PROCEDURAL-LEGAL ASPECTS OF INPATIENT AND OUTPATIENT FORENSIC PSYCHIATRIC EXAMINATION	1533
Volodymyr V. Marchenko, Inna I. Kilimnik, Alla V. Dombrowska IMPLEMENTATION OF DIGITAL TECHNOLOGIES IN HUMAN RIGHTS TO HEALTHCARE	1539
Igor Rakoczy, Marzena Ocetek, Marzena Wiatr, Patrycja Zięba, Michał Panek, Piotr Kuna NON-NECROTIZING INFLAMMATORY GRANULOMAS (N-NGS) IN THE COURSE OF INFLAMMATORY BOWEL DISEASE – IMMUNOLOGY AND CLINICAL MANIFESTATION OF INTESTINAL AND RESPIRATORY N-NGS	1545
Viktor V. Horodovenko, Vitalii M. Pashkov, Larysa G. Udovycha INTERNATIONAL LEGAL INSTRUMENTS IN THE FIELD OF BIOETHICS AND THEIR IMPACT ON PROTECTION OF HUMAN RIGHTS	1554
Olena V. Uvarkina, Olena M. Bieliaieva, Petro M. Skrypnykov, Yuliia V. Lysanets, Liudmyla M. Shylkina, Tetiana A. Khmil THE SCIENTIFIC AND PEDAGOGICAL HERITAGE OF DOCTOR N.I. PIROGOV	1561
Małgorzata Paszkowska NEW PHYSIOTHERAPIST STATUS IN THE POLISH HEALTH CARE SYSTEM	1567
Tomasz Torliński, Lucas Rakasz, Barbara Wysota, Marcin Czyż, Catherine Snelson AN INTERDISCIPLINARY APPROACH TO THE MANAGEMENT OF CRITICALLY ILL PATIENTS DURING COVID-19 PANDEMIC; AN EXPERIENCE OF A UNIVERSITY HOSPITAL IN ENGLAND	1576

CASE STUDIES

Olga Możejka, Michał Wojewódzki, Diana Wiligórska, Natalia Wiligórska, Robert J. Gil, Jacek Bil CONSERVATIVE TREATMENT AS A THERAPEUTIC OPTION FOR PENETRATING AORTIC ULCER OF THE AORTIC ARCH IN A PATIENT WITH BICUSPID AORTIC VALVE	1580
Igor Alexander Harsch, Marcin Skiba, Eric Lopatta, Peter Christopher Konturek HYDROXYCHLOROQUINE IS NO MIRACLE CURE FOR COVID-19 INFECTION –IMAGING AND CLINICAL COURSE IN AN ELDERLY FEMALE	1583

EDITORIAL ARTICLE

METHODS OF SPECTRAL DIAGNOSTICS IN MODERN PREVENTION OF ONCOLOGICAL DISEASES

DOI: 10.36740/WLek202007101

Jakub Adamczyk¹, Dominik Sieroń², Karolina Sieroń³, Aleksander Sieroń⁴, Ewa Kucharska⁵^{1,4}JAN DŁUGOSZ UNIVERSITY IN CZESTOCHOWA, FACULTY OF HEALTH SCIENCES, CHAIR OF HEALTH SCIENCES AND PHYSIOTHERAPY, CZESTOCHOWA, POLAND²INSTITUTE OF RADIOLOGY, TIEFENAU HOSPITAL, INSELGROUP, UNIVERSITY OF BERN, BERN, SWITZERLAND³SCHOOL OF HEALTH SCIENCES IN KATOWICE, MEDICAL UNIVERSITY OF SILESIA IN KATOWICE, DEPARTMENT OF PHYSICAL MEDICINE, CHAIR OF PHYSIOTHERAPY, KATOWICE, POLAND⁵DEPARTMENT OF GERONTOLOGY, GERIATRICS AND SOCIAL WORK JESUIT UNIVERSITY IGNATIANUM IN KRAKÓW, KRAKOW, POLAND

ABSTRACT

This paper contains structured information on photodynamic diagnostics. Photodynamic diagnostics is a young diagnostic modality used in the detection of pre-neoplastic and very early neoplastic lesions. A characteristic feature of the presented method is its completely non-invasive nature and thus the possibility of multiple repetitions at the same patient. This is very important in modern health care and in preventive measures.

Aim of the paper: The article aims to present technical and diagnostic possibilities of a photodynamic method as one of the possible modalities of screening diagnostics in patients with ambiguous clinical picture of early neoplastic lesions.

KEY WORDS: early cancerous changes, spectral analysis, spectrum, lasers, LED diodes, photodynamic diagnostics

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In the 20th century we witnessed a rapid civilization development based mainly on the development of new technologies. The 21st century will be based on the development of interdisciplinary discoveries from the border of broadly defined physics, chemistry and biology. Undoubtedly, we will see most discoveries in space technologies and medicine. An example in the field of medicine is photodynamic diagnostics intensively developed in recent years. This is an excellent example of using knowledge in the field of physics, biology and biochemistry to develop a modern technique of medical imaging diagnostics used in visualizing early cancerous lesions. This method may be an important diagnostic branch with high screening utility because it allows to quickly and in a non-invasive way determine the nature of the examined lesion in patients with suspected neoplastic lesions. This is important in the current health care system where low-cost, non-invasive methods for mass diagnostics of patients are sought.

The first works related to photodynamic diagnostics were done already at the turn of the 19th and 20th centuries. At the time, interest was given to the fact that the lighting of the slipper animalcule (*Paramaecium Caudatum*) caused a lethal effect at low acridine levels. In comparative tests using a similar concentration of the dye in the solvent, but without access to light, this effect did not occur [1].

The first use of the photodynamic method took place in 1903. Eosin was then used as a photosensitizer. In 1911

hematoporphyrin was used as a photosensitizer. In 1924, tumor fluorescence was demonstrated in mice based on the action of endogenous porphyrins. Also, that year, the accumulation of exogenous hematoporphyrin in cancer cells was more than 4 times higher than in healthy cells. The first commercial photosensitizer was created in the 1960s under the name HpD and was a mixture of various porphyrin derivatives. At the same time, a number of studies were carried out on the optical properties of tissues and cells, as well as light itself, which is subject to complex physical phenomena in the cell. Light as an electromagnetic wave in a cell is subject to transmission and absorption. There is also reflection, Raman dispersion and elastic dispersion. Energy is transferred by fluorescence or phosphorescence [2].

In the course of experiments, the usefulness of this method has been demonstrated for the diagnosis of early neoplastic lesions based on fluorescence by differentiating neoplastic tissues from normal (Fig. 1).

Photodynamic diagnostics is a non-invasive diagnostic method. It allows one to analyze the observed lesion in real time in the visible light range. This means that it allows one to perform an analysis on the patient's skin at any place and time. It also means the ability to analyze patient's internal structures that can be diagnosed using speculum and endoscopes. Thus, in addition to dermatology, this method is constantly being developed within gastroenterology,

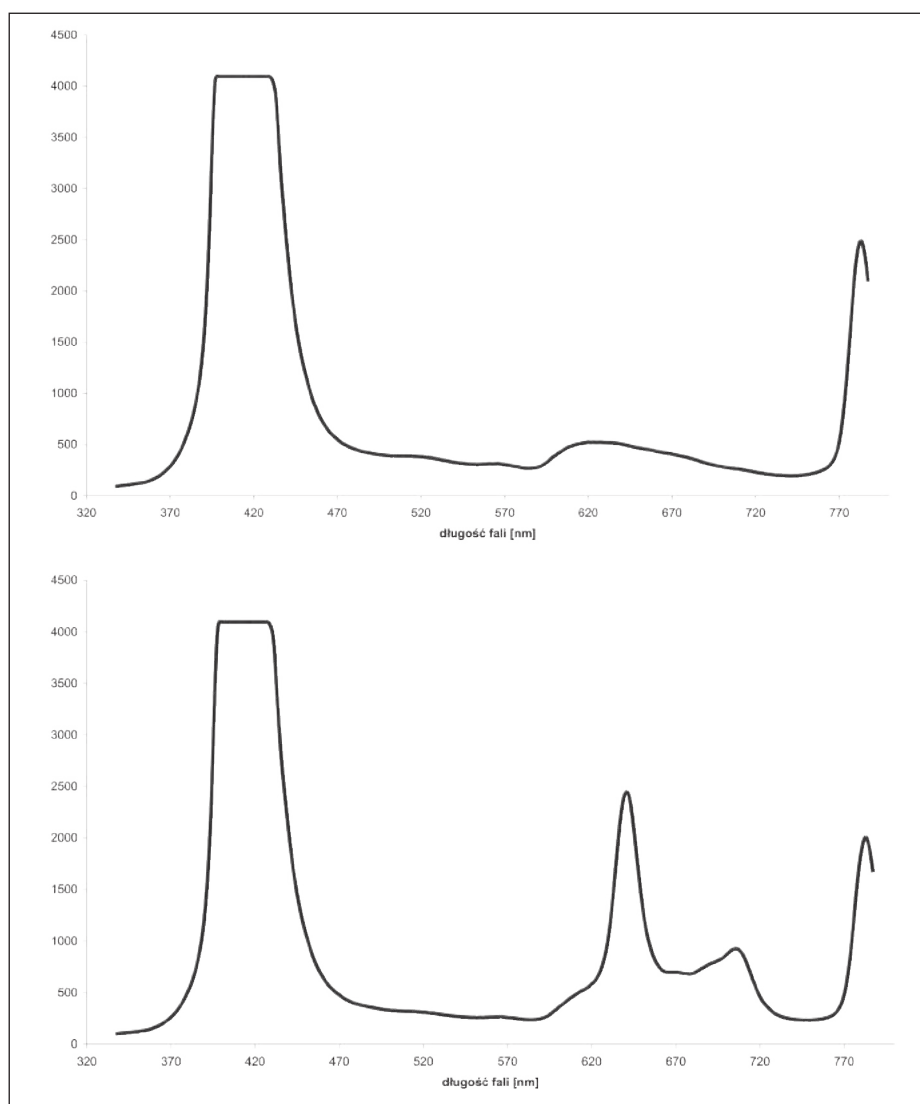


Fig. 1. Spectrum of healthy tissue and neoplastic tissue (own material).

gynecology, urology and pulmonology. A characteristic feature of this method, due to the use of visible light, is the possibility of repeated analyzes of the same area. This enables not only very accurate identification of the dysplastic area but also monitoring of the treatment process.

The method is based on the phenomenon of light emission by tissues. The affected tissue contains protoporphyrin, which emits light of a different, defined wavelength as a result of the light brought into it. For this phenomenon to occur, the observed tissue is illuminated with blue light. The whole process must take place in the so-called optical skin window. It is the range of visible light that penetrates deepest into the tissue. The maximum transmittance used for photodynamic therapy is in the range of 800-1200 nm. Dynamic diagnostics use a shorter length ensuring light penetration to a depth of 0.5-2 mm. This range was specified in the 400 – 500 nm range. For precise selection of the wavelength of light and at the same time ensuring adequate energy, the light generated by lasers is used. Laser light provides strictly defined and repeatable physical parameters. Most often blue light with a wavelength of 405 nm is used to induce

autofluorescence. A certain difficulty, however, is still the high cost of devices generating the laser beam. The solution to this problem was the use of xenon or mercury light sources equipped with appropriate barrier filters. This solution, much cheaper than a laser, however, generated large amounts of heat, difficult to eliminate in treatment room conditions. In addition, strictly defined and short life span of this type of light sources generated high operating costs. The applied optical filters allowed for obtaining a heterogeneous wavelength. In the case of lasers, the wavelength was strictly defined, while in the case of optical filters, the wavelength was in the range of 20-80 nm next to the required peak. Digital cameras are the indispensable equipment for autofluorescent diagnostic systems. Modern cameras are miniature and light with high resolution transducers. In addition, dedicated software allows the use of proprietary measurement methods to obtain specific numerical values in the area of the observed and analyzed area. This makes it possible to compare the obtained images and thus creates broad analytical possibilities and monitoring of the treatment process itself [3].

The fastest development of photodynamic diagnostics took place in pulmonology. Bronchial tree imaging during standard bronchoscopic examination has been perfectly developed. Dedicated diagnostic systems have been created to enable standard bronchoscopy and subsequent testing using the possibility of tissue autofluorescence. Endoscopic examinations carried out so far in white light did not always give a full diagnostic response specifying the nature of the observed lesion. The result of image analysis was often not clear-cut. In addition, the observed area suspected of dysplastic lesions at a very early stage of development in white light is almost imperceptible. A reliable diagnosis requires a biopsy specimen for histopathological analysis and the final diagnosis is made on the basis of this examination. Often in the case of a white light test, the collection of a specimen from the affected area is unsuccessful. Autofluorescence diagnostics fully solves this problem. The doctor conducting tests in blue light, through the appropriate software of the whole device, sees healthy tissues as green areas. All irregularities are shown in shades of red. The intensity of the red color depends largely on the stage of the tumor formation process. This fact undoubtedly constitutes the superiority of the proposed solution over classic endoscopic techniques performed in white light. In the absence of certainty as to the observed area after switching to blue light, the examining physician can clearly see where from which the histopathological examination should be taken. Thus, using this method results in less burden on the patient himself. No further endoscopic tests are needed to burden the health care system, but also to cause a great feeling of patient discomfort. In the field of pulmonology, clinical tests and diagnostic devices available on the market have been used in the diagnosis of early neoplastic lesions such as bronchopulmonary cancer. Autofluorescence diagnostics is especially useful in patients whose cancer cells were found in sputum, but imaging tests such as chest X-ray or classic bronchoscopy did not give a definitive diagnostic result. In pulmonology, autofluorescent diagnostics are used in patients qualified for surgery, after the surgery to monitor the extent and possible recurrence as well as to assess the pathomorphological assessment of the upper respiratory tract mucosa [4].

In addition to pulmonology, the second application of autofluorescence diagnostics is its use in assessing colorectal lesions. During the colonoscopy, the image of the mucosa is assessed. This allows one to unambiguously assess changes in ulcerative colitis and colitis that often last for several years. High diagnostic utility has been demonstrated in the assessment of colorectal polyps with diagnosis such as adenoma villosum and tubulovillous. In the field of preventive medicine, the presented method is extremely useful in periodic assessment in patients after previous resection and anastomosis of the lower gastrointestinal tract as a result of colorectal cancer or surgery as a result of complications after long-term inflammation of the intestinal walls. This is especially true for patients with suspected recurrent tumorigenesis revealed in clinical history or biochemical tests. Prophylactic treatment also

applies to genetically burdened patients, who naturally have a significantly higher risk of developing cancer [5].

Also, the upper gastrointestinal tract can be diagnosed using autofluorescent methods. It is a method widely used in assessing lesions within the esophagus and the stomach. It allows you to search for dysplastic lesions within the Barret's esophagus, significantly facilitating the accurate collection of specimens for histopathological examination. It also allows the assessment of benign ectopic or papillary lesions within the mouth and esophagus [6].

Available research results show the great potential of photodynamic therapy in urology. This applies in particular to bladder disorders that require very high precision when collecting specimens for histopathological examination. Very high usefulness in imaging urinary tract lesions has been demonstrated. This method can be used as a screening test with very high sensitivity while maintaining minimal invasiveness in the field of imaging of the bladder and ureters. Indication for cystoscopy using fluorescence imaging is the suspicion of intraepithelial bladder cancer (in situ) in patients with a history of hematuria and inflammation of the urinary tract resistant to anti-inflammatory drugs for over 3 months. Patients after transurethral resection or neoplastic foci within the bladder wall may also be referred for the purpose of assessing the radicality of the procedure and for assessing new or recurrent neoplastic foci [7].

Photodynamic diagnostics is also used in gynecology in the early stages of vulvar and cervical lesions. An indication for carrying out the test using this technique is to obtain an abnormal cytological test result of a swab taken from the cervix showing endothelial features of a high-grade lesion. A vulva analysis is also performed after radical treatment as a result of a neoplastic lesion and in patients with suspected relapse. Scleroderma and lichen sclerosis with confirmed dysplasia are assessed. The effectiveness of autofluorescence studies in imaging of lesions in gynecology in women with confirmed human papillomavirus infection has also been demonstrated, which did not show clinical lesions but had confirmed dysplasia in cytological examination [8].

The importance of photodynamic diagnostics has been demonstrated in dermatology. It is conditioned by its completely non-invasive character and practically unlimited repetition. Subassemblies for the construction of autofluorescent diagnostics systems for dermatology are characterized by simplicity and do not require engineering studies necessary in the case of endoscopy to obtain the possibility of integrating cameras of different manufacturers, with many models and endoscope mounting systems, which must also be combined with appropriate light sources. The use of the LIF method in the diagnosis of skin cancers is conditioned by a high concentration of nicotinamide adenine nucleotide redox. A number of lesions such as melanoma, other skin cancers, birthmarks, seborrheic warts and psoriasis have similar fluorescence. Only melanoma has much weaker fluorescence compared to healthy skin. The location of skin cancer, especially basal and squamous cell carcinoma, is greatly facilitated by the topical use of a photosensitizer. Neoplastic skin lesions can occur in every

layer. Basal cell carcinoma accounts for about 70-80% of all skin cancers. In second place is squamous cell carcinoma, the incidence of which is 20-25%. Unlike squamous cell carcinoma, basal cell carcinoma does not usually lead to metastasis, but it can infiltrate deeper tissues. An important condition for curing any cancerous lesion is its quick and reliable diagnosis. The detection of basal cell carcinoma, despite being easily histopathologically diagnosed, is often delayed for many years, usually due to the patient. Usually, the lesion is neglected, and its variability of the image diminishes the alertness of the doctor and the patient [9].

Nowadays, new diagnostic systems for localization and often identifying early cancerous lesions are raising the expectations of medicine. Interest in these methods depends on their high sensitivity and resolution compared to traditional diagnostic imaging methods such as computed tomography, magnetic resonance imaging or ultrasound. The most modern optical diagnostic methods, unlike histopathological tests, do not require the collection of biological material. Exciting radiation, often from the visible light range, is led to the examined lesion by optical fibers and emission spectra are received and analyzed in real time. An additional advantage is the fact that the studied areas can be analyzed many times. In recent years there has been an increase in interest in tissue autofluorescence. Only in few diagnostic centers in the world this method is generally available, because it requires very expensive optical diagnostic equipment and highly qualified interdisciplinary staff.

Therefore, spectral analysis is a support for treatment planning, often informing about the biochemical structure of the observed lesion. Specialized algorithms and models developed for tri-modal laser spectroscopy are helpful here.

Tri-modal laser spectroscopy is currently the most developed technique for tissue spectral analysis. The Massachusetts Institute of Technology is constantly at the forefront of ongoing work. This method provides information on the cellular and tissue structure and characterizes the biochemical structure of the examined tissue. It is based on the phenomena of dispersion, fluorescence and backscatter. Although this method is still in the testing and analysis phase, it already gives information characterizing and identifying the examined lesion [10].

Spectral studies contribute to faster and more accurate analysis of the observed lesion suspected of developing cancer. It seems reasonable to say that this diagnosis should be in addition to commonly available diagnostic tests.

The paper presents examples of disease entities for which the photodynamic method is applicable. According to the current standard, the definitive test is a histopathological test. However, in early neoplastic lesions, the area of the lesion is often unnoticeable when examined in white light. The optimal situation would be the possibility of conducting a fluorescent examination in all suspected patients, thus guaranteeing a single procedure and removal of a section from the pathologically changed area.

Efficacy in the treatment, for example, squamous cell carcinoma in situ is high and oscillates in the range of 75-92%

[11, 12]. The same is true for basal cell carcinoma. In the case of detection of endothelial cervical neoplasia, the sensitivity of the method was 60% and the specificity 90% [13].

Photodynamic diagnostics enables quick and correct formulation of an unambiguous diagnosis, which significantly shortens the treatment process. Thanks to this method, it is possible to collect a slice from very early neoplastic lesions or ambiguous lesions in the clinical picture of recurrence processes. This applies to any changes that can be seen on the skin or with the help of endoscopic instrumentation [14].

The correct image of skin or mucosa may mask pathological changes in some cases. The diagnostic process of these changes is completed very quickly if it is possible to verify them. This is of great importance not only for the doctor, but also for the patient. The current economization of health care forces to look for increasingly cheaper and faster diagnostic methods that allow one to start therapeutic procedures faster or a faster, negative verification favorable for the patient.

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Conflict of interest

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ORIGINAL ARTICLE

STRUCTURAL REORGANIZATION OF THE RATS' SUBMANDIBULAR GLANDS ACINI AFTER THE INFLUENCE OF 1% METHACRYLATE

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Introduction: Polymeric substances, which are used in medicine, can lead to the development of pathological conditions. Therefore, in toxicological evaluation of polymeric materials for medical purposes the primary task is the creation of the appropriate adequate methods of study.

The aim: To study the acini of the rats' submandibular salivary glands in normal condition and after exposure of 1% methacrylate.

Material and methods: Were studied 50 white rats during the influence of 1% methacrylate on the mucosa of the oral cavity. Histological sections of the rats' submandibular salivary glands in normal condition, on day 14 and 30 of experiment were studied using the UMPT – 7 ultramicrotome of Sumy PA "Selmi".

Results and conclusions: The morphometric study has established that in control group the values of the outer diameter of the submandibular glands' acini, the diameter of the lumen and the height of the acini epithelial cells was $36,27 \pm 2,17 \mu\text{m}$, $9,47 \pm 0,63 \mu\text{m}$ and $14,18 \pm 1,05 \mu\text{m}$, respectively. Histological specimens showed that the acini cells had a prominent basophilia of the cytoplasm and the basally located nucleus. Numerous large homogeneous granules were located in the apical pole of the cells. After influence of the 1% methacrylate there is an increase in the activity of secretory cells, which is confirmed by an increase in the height of epitheliocytes by 25.04% and is a compensatory methacrylate reaction. The increase in salivation further leads to complete depletion of the secretory apparatus of the seromucosal cells, which is confirmed by a decrease in the outer and inner diameters with a decrease in the height of the mandibular salivary glands acini cells by 24.40%.

KEY WORDS: salivary glands, rats, acini, epithelial cells, 1% methacrylate

Wiad Lek. 2020;73(7):1318-1322

INTRODUCTION

Polymeric materials and substances, that migrate from polymeric materials, are used in medicine, and they can lead to the development of various pathological conditions. Therefore, in toxicological evaluation of polymeric materials for medical purposes the primary task is the creation of the appropriate adequate methods of study, which include the analysis of such factors as the nature and duration of contact of the polymeric product with the human body [1]. However, despite the disadvantages, methacrylates have been widely used in dentistry, as the base of dentures, due to their general using [2, 3]. The toxic effect can be caused by the methyl ester of methacrylic acid. However, its toxic properties appear only at high doses [4]. Many scientific articles are devoted to the study of the effect of methacrylate monomer on the mucosa of the oral cavity [5, 6], though few of them are devoted to the study of the state of organs located outside the oral cavity after exposure to methacrylic acid.

It has been proved by evidence that patients, who wear dentures, often complaints of xerostomia. In this case, the major salivary glands must compensatory increase secretion to provide adequate moistening of the oral cavity mucosa [7]. The major salivary glands are composed of the secretory acini and the system of excretory ducts [8, 9]. They consist

of various glandular cells that are programmed to synthesize completely different products of secretion [10, 11]. Investigating the histochemical characteristics of the structure of the acinar unit of the human major salivary glands, the researchers have found that the acinar units of the salivary glands are represented by two differentiated types of the secretory cells. The specificity of their differentiation is manifested by the secretion of mucus and protein [12, 13], which is crucial for the qualitative composition of saliva, which, subsequently, will affect the condition of the human oral cavity, dental and somatic health in general; therefore, studies in this field are of great practical importance [14, 15].

THE AIM

To determine the changes in the structural elements of the acini of the rats' submandibular salivary glands in normal condition and after exposure of 1% methacrylate.

MATERIALS AND METHODS

Were studied white male rats (n= 50; controls: n=10; experimental animals: n=40). The mucosa of the oral cavity of rats was exposed by 1% methacrylate for 30 days [5]. The animals were killed on day 14 and 30. The fragments of submandib-

Table 1. The dynamics of the changes of morphometric parameters of the acini of the submandibular glands (in μm)

Acini	The outer diameter	The diameter of the lumen	The height of the epithelial cells
Control group (n=5)	$36,27 \pm 2,17$	$9,47 \pm 0,63$	$14,18 \pm 1,05$
Day 14 (n=5)	$37,33 \pm 2,96$	$8,83 \pm 0,72$	$17,73 \pm 1,11^*$
Day 30 (n=5)	$28,99 \pm 2,14^{**}$	$7,89 \pm 0,82^*$	$10,72 \pm 0,98^{**}$

Note: * – $P < 0,05$ compared with control group;

** – $P < 0,05$ compared with previous period of experiment.

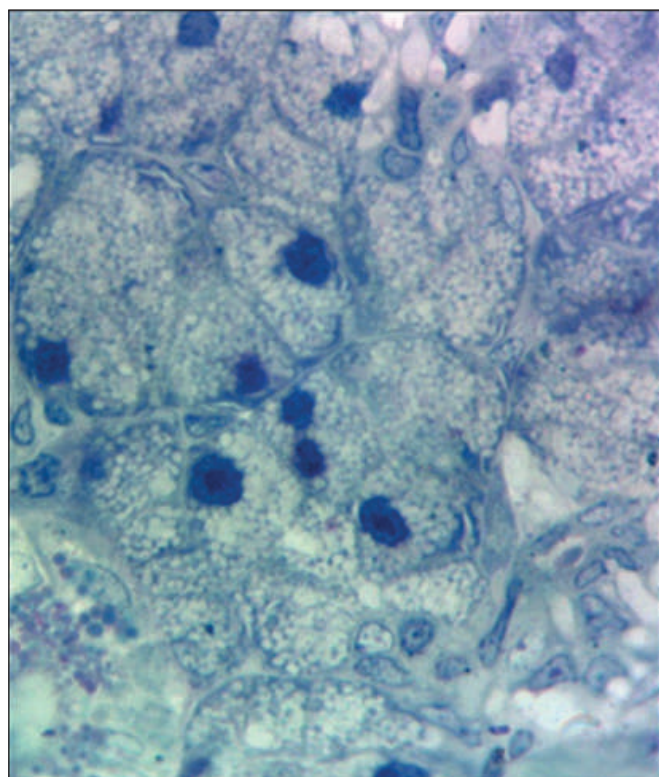


Fig. 1. Acini of the rats' submandibular gland on day 14 after the effect of 1% methacrylate. Specimen. Polychrome staining. x1000.

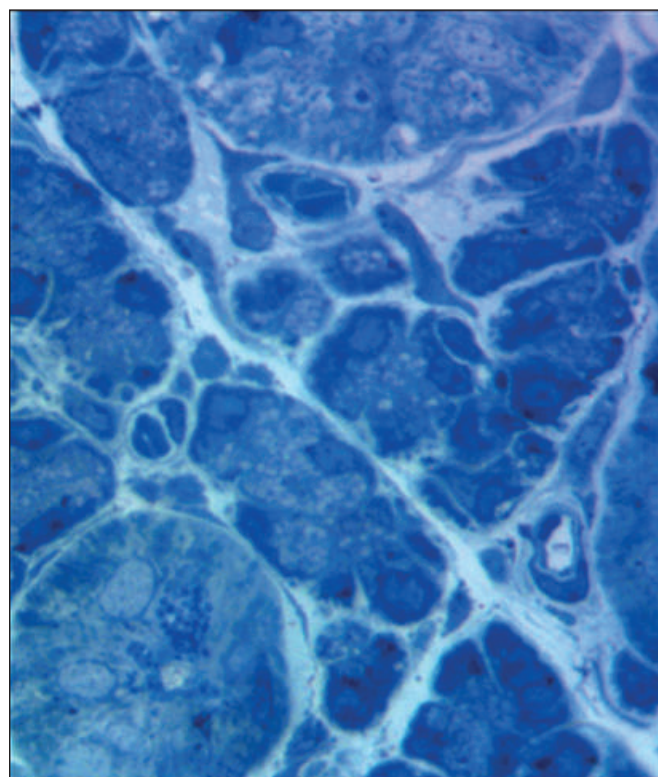


Fig. 2. Lobules of the rats' submandibular glands on day 30 of the experiment after the effect of 1% methacrylate. Specimen. Stained with Methylene blue. x1000.

ular glands were embedded into epon-812 [16]. Histological sections were made using the UMPT – 7 ultramicrotome of Sumy PA "Selmi" and stained with methylene blue and polychrome stain [17]. Morphometric study was doing by the Biorex-3 BM-500T microscope with digital DCM 900 microphoto-head with software, adapted to these studies. Conventional statistical methods have been used for statistical processing of the morphometric data and quantitative analysis by Microsoft Excel software [18].

Experiments have been carried out in compliance with the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [19].

RESULTS AND DISCUSSION

The morphometric study [20] has established that in rats of control group the values of the outer diameter

of the acini of the submandibular glands, the diameter of the lumen and the height of the acini epithelial cells was $36,27 \pm 2,17 \mu\text{m}$, $9,47 \pm 0,63 \mu\text{m}$ and $14,18 \pm 1,05 \mu\text{m}$, respectively (Table 1).

Histological specimens of the submandibular gland showed that the pyramidal glandular cells of the acini had a prominent basophilia of the cytoplasm and the basally located nucleus. Numerous large homogeneous granules with products of secretion were located in the apical pole and in central part of the cells.

During the study, after exposure of 1% methacrylate for 14 days, the outer and inner diameter of the acini were $37,33 \pm 2,96 \mu\text{m}$ and $8,83 \pm 0,72 \mu\text{m}$, respectively, which did not differ significantly from the same parameters of the control group of rats. The height of the acini epithelial cells of the submandibular glands lobules was $17,73 \pm 1,11 \mu\text{m}$, which was by 25.04% significantly more than its value in the control group of animals. On day 14 of the study in

the cytoplasm of the epithelial cells, an increased optical density was observed due to increase in secretory granules that occupied the cytoplasm of the cells (Fig. 1).

On day 30 of the experiment morphometric study has established that the diameter of the outer acini of the lobules of the rats' submandibular glands was $28.99 \pm 2.14 \mu\text{m}$ that was significantly lower by 22.34 % than the values on day 14 of the experiment and by 20.07 % lower than the value in the control group.

No significant difference in the values of the diameter of the lumen compared to previous time period of the experiment was detected; however, they were by 20.07 % lower than the values in the control group, accounting for $7.89 \pm 0.82 \mu\text{m}$. The height of the epithelial cells was $10.72 \pm 0.98 \mu\text{m}$ that was significantly lower by 39.54 % than the value on day 14 of the experiment after the effect of 1 % methacrylate and by 24.40 % than the mean values in the control group of rats.

The cytoplasm of the acinar epithelial cells was heterogeneous; secretory granules of different optical density were detected that was caused by the presence of protein and carbohydrate components in them. Thickening of the interstitial connective tissue and prominent intercellular fissures were visualized. The lumens of the blood vessels of the microcirculatory bed were dilated (Fig.2).

Thus, the comparison of our results with previous studies has shown that the acini of the rats' submandibular glands after exposure of 1 % methacrylate had unidirectional relationship with the acini of the minor submandibular salivary glands, which on day 30 showed a decrease in height of the epithelial cells by 50%, compared to day 14 of the experiment, and by 25.3 % compared to control group of rats [7].

The height of the epithelial cells of the acini of the submandibular salivary gland decreased by 39.54%, compared to the previous time period of the experiment, and by 24.40% compared to the control group of rats that was primarily caused by location of the minor salivary glands in the thickness of the lamina propria of the hard palate mucosa and the direct influence of the denture base monomer on them.

The study of the structure of the lobules of the rats' submandibular salivary glands in stimulation of the sympathetic and parasympathetic units has revealed adrenaline-related significant enlargement of the outer diameter by 12.65%, an increase in the height of epithelial cells by 14.86% with a decrease in the diameter of the lumen of the acini of the lobules of the rats' submandibular glands that is a morphological evidence of increased secretory activity of seromucosal cells with predominance of carbohydrate content in the secretory granules of the acini in response to stimulation [21, 22]. Acetylcholine injection lead to the significant decrease of the outer diameter by 6.07%, a significant decrease of diameter of the lumen by 58.89%, an increase of height of the epithelial cells, compared to the control group during morphometric study. Consequently, it has been found that stimulation of cholinoreceptors led to hypersalivation with increasing functional activity of

all secretory components and was manifested by the decrease in the absolute size of the granules in the mucosa secreting cells and their tinctorial properties. This changes correspond to the changes after the effect of methacrylate on day 14 of the experiment with an increase in secretory activity in response to the effect of exogenous factor, caused by compensatory mechanisms to the impact of the stimulus, with hypersalivation, unlike a single action and response of the parenchyma to stimulation of cholino- and adrenoreceptors. Subsequently, the effect of methacrylate leads to a decrease in secretory activity due to the depletion of cells of the acini of the lobules of the submandibular glands [23, 24, 25].

It is very interesting to analyze the functional activity of the acini and their structural changes in stimulation of the rats' salivary glands by platyphyllin and proserin [26, 27, 28]. No significant changes ($p < 0.05$) in the value of the height of the epithelial cells have been noted after their impact, compared to controls, in contrast to the effect of methacrylate. However, the value of the lumen diameter in stimulation with platyphyllin decreased by 45.2%, with no significant changes in values in control group after influence of proserin. After the effect of 1% methacrylate, the values of the lumen diameter were by 20.07% lower, compared to the values in controls. The changes were caused by the warming of the interstitium after influence of platyphyllin, due to the stimulation of cholinoreceptors of parasympathetic postganglionic fibers and increase in the transport of juxtacellular fluid, which is the morphological feature of increased functional activity of seromucous cells of the acini in response to stimulation during the experiment. The established increase of the secretory activity of the epithelial cells in response to methacrylate occurs only in the middle of the experiment due to the enhanced activity of the secretory apparatus of cells and, as a consequence, increased the number of secretory granules (Fig. 1).

The ethanol influence on the acini of the rats' submandibular glands in the middle of the experiment showed a decrease of the outer and inner diameters by 7.57% and 14.61%, respectively; the height of the epithelial cells decrease by 6.11% compared to control with a tendency to decrease throughout the experiment, due to inhibition of secretion, in contrast to the effect of methacrylate, where the height of the epithelial cells significantly increased on day 14 of the experiment by 25.04%, due to the compensatory increase in secretion. At the end of the experiment, the height of the epithelial cells decreased by 24.40% compared to the control group of animals, which indicates the depletion of secreting cells due to the permanent toxic effect of methacrylate; a decrease by 21.44% of the height of the epithelial cells induced by ethanol, compared to the control group, occurs due to dystrophic changes and restructuring of the secretory apparatus to carbohydrate synthesis [29, 30].

CONCLUSIONS

1% methacrylate influence on the rats' submandibular salivary glands causes the increase of secretory activity of

glandular cells of the acini on day 14 of the experiment. It is confirmed by an increase of the height of the epithelial cells by 25.04% and is a compensatory reaction of the salivary glands to the action of methacrylate. This is accompanied by hypersalivation, which subsequently leads to complete depletion of the secretory apparatus of the seromucous cells of the acini and is confirmed by a decrease in the outer and inner diameters with decrease in height of epithelial cells of the acini of the submandibular salivary glands by 24.40%, due to prolonged effect of 1% methacrylate on the oral cavity mucosa.

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ORIGINAL ARTICLE

CZYNNIKI RYZYKA ZŁOŚLIWOŚCI ZMIAN OGNISKOWYCH TARCZYCY ZWERYFIKOWANYCH W BIOPSJI ASPIRACYJNEJ CIENKOIGŁOWEJ CELOWANEJ JAKO "PODEJRZENIE NOWOTWORU PĘCHERZYKOWEGO"

RISK FACTORS OF MALIGNANCY IN PATIENTS WITH FINE NEEDLE ASPIRATION BIOPSY RESULTS INTERPRETED AS "SUSPICIOUS FOR FOLLICULAR NEOPLASM"

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STRESZCZENIE

Wstęp: Rozpoznanie cytologiczne „podejrzenie nowotworu pęcherzykowego” stawiane jest w około 10% biopsji aspiracyjnej cienkoigłowej celowanej (BACC) zmian ogniskowych tarczycy. Kategoria obejmuje: gruczolaka pęcherzykowego, raka pęcherzykowego, wariant pęcherzykowy raka brodawkowatego oraz ich warianty oksyfilne. Do chwili obecnej nie wprowadzono do praktyki klinicznej narzędzia diagnostycznego, za pomocą którego można wykluczyć obecność nowotworu złośliwego i stąd też część chorych z podejrzeniem nowotworu pęcherzykowego tarczycy wymaga leczenia operacyjnego w celu postawienia ostatecznego rozpoznania.

Cel pracy: Wyodrębnienie czynników ryzyka raka tarczycy u chorych ze zmianami ogniskowymi lub guzami tarczycy zweryfikowanymi w BACC jako „podejrzenie nowotworu pęcherzykowego”.

Materiały i metody: Do badania zakwalifikowano kolejnych 110 chorych operowanych od stycznia 2016 do marca 2020 z powodu podejrzenia nowotworu pęcherzykowego tarczycy. Kryteria włączenia stanowiły: wynik BACC – klasa IV według klasyfikacji Bethesda, brak ewidentnych cech złośliwości guza, takich jak: naciekanie okolicznych tkanek, podejrzenie przerzutów do węzłów chłonnych bądź przerzutów odległych stwierdzonych w badaniu klinicznym lub w przedoperacyjnych badaniach obrazowych.

Wyniki: U 18 chorych (16,3%) rozpoznano raka tarczycy. Najczęstszym podtypem okazał się rak brodawkowaty (66,6%). Spośród zmian łagodnych (92 chorych) dominował gruczolak pęcherzykowy (49%). Nie stwierdzono istotnej statystycznie zależności pomiędzy rakiem tarczycy a: płcią, średnicą guza, wiekiem, sumarycznym wynikiem klinicznych i ultrasonograficznych cech zwiększonego ryzyka złośliwości zmian ogniskowych, obecnością choroby Hashimoto oraz wynikiem BACC *podejrzenie nowotworu z komórek Hurthle’a*.

Wnioski: U chorych z wynikiem cytologicznym *podejrzenie nowotworu pęcherzykowego tarczycy* uzyskanym w wyniku BACC nie występują kliniczne czynniki pozwalające na odstępianie od leczenia operacyjnego z uwagi na statystycznie mniejsze ryzyko nowotworu złośliwego.

SŁOWA KLUCZOWE: rak tarczycy, podejrzenie nowotworu pęcherzykowego tarczycy, podejrzenie nowotworu z komórek Hurthle’a tarczycy, gruczolak pęcherzykowy tarczycy

ABSTRACT

Introduction: Approximately 10% of fine needle aspiration biopsy (FNAB) of thyroid nodules may be verified as “suspicious for follicular neoplasm”; this category involves follicular adenoma, follicular carcinoma, follicular variants of papillary carcinoma and subclass “suspicious for Hurthle cell neoplasm”. At present, there is no diagnostic tool to discriminate between follicular adenoma and cancer. Most patients are required surgery to exclude malignant process.

The aim: To define factors correlating with risk of malignancy in patients with FNAB of thyroid focal lesions and nodules verified as Bethesda tier IV.

Materials and Methods: In this study 110 consecutive patients were included. All patients were operated because of FNAB result “suspicious for follicular neoplasm” of thyroid gland at a single institution from January 2016 until March 2020. From this set, six specific categories were defined and the clinical records for patients were collected: sex, age, presence of oxyphilic cells, diameter of the tumour, presence of Hashimoto disease, aggregate amount of clinical and ultrasonographic features of malignancy according to ATA.

Results: In 18 patients (16,3%) thyroid cancer occurred. Most frequent subtype turned out to be papillary cancer (66,6%). In group of benign lesion (92 patients) predominance of follicular adenoma was disclosed – (49%). Age, gender, tumour diameter, aggregate amount of clinical and ultrasonographic factors, presence of Hashimoto disease and fine needle aspiration biopsy result *suspicious for Hurthle cell neoplasm* did not correspond to increased risk of malignancy.

Conclusions: In patients with FNAB results classified as Bethesda tier IV there are no reliable clinical features associated with low risk of malignancy and surgery should be considered in every case as most appropriate manner to exclude thyroid cancer.

KEY WORDS: follicular adenoma, thyroid cancer, suspicious for follicular neoplasm, suspicious for Hurthle cell neoplasm

SKRÓTY

ATA – Amerykańskie Towarzystwo Tyreologiczne (*American Thyroid Association*)

NKW – nerw krtaniowy wsteczny

RT – rak tarczycy

RBT – rak brodawkowaty tarczycy, wariant klasyczny

RPT – rak pęcherzykowy tarczycy;

WPRBT – wariant pęcherzykowy raka brodawkowego tarczycy

ChH – choroba Hashimoto

BACC – biopsja aspiracyjna cienkoigłowa celowana

USG – badanie ultrasonograficzne

TBSRTC – klasyfikacja cytologiczna biopsjowanych zmian ogniskowych tarczycy (*The Bethesda System for Reporting Thyroid Cytopathology*)

WSTĘP

Guzki tarczycy stanowią powszechną patologię tego narządu i są najczęstszym powodem konsultacji endokrynologicznej. Według badań autopsyjnych u ponad 50% populacji stwierdza się przynajmniej 1 zmianę niemą klinicznie o średnicy przekraczającej 1 cm, natomiast odsetek guzów wykrywanych podczas badania palpacyjnego waha się w przedziale 4-7% [1, 2]. Czułość badania ultrasonograficznego (USG) przewyższa badanie kliniczne i pozwala rozpoznać zmiany ogniskowe nawet u 67% populacji [3]. Kolejne miejsce w algorytmie diagnostycznym zajmuje biopsja aspiracyjna cienkoigłowa celowana (BACC). BACC charakteryzuje się dużą dokładnością i pozwala zakwalifikować zmianę ogniskową jako łagodną bądź złośliwą z ryzykiem złośliwości odpowiednio <5% oraz >96% [4]. W Polsce wynik badania cytologicznego jest raportowany według systemu Bethesda (TBSRTC – *The Bethesda System for Reporting Thyroid Cytopathology*), który wyróżnia 6 kategorii, przypisując każdej określone ryzyko złośliwości (Tab. I) [5].

Odsetek wyników BACC, których rezultat nie pozwala na wybór optymalnej ścieżki postępowania ze względu na zbyt niską czułość, zawiera się w przedziale 0,4–40,7% (średnio 20%) [6-8]. Doskonały przykład takiej biopsji zwanej „nieokreśloną” stanowi kategoria IV – podejrzenie nowotworu pęcherzykowego tarczycy odpowiadając za 1,2–25,3% BACC, średnio 10%. W tej kategorii mieszczą się następujące rozpoznania histopatologiczne – gruczolak pęcherzykowy, rak pęcherzykowy oraz wariant pęcherzykowy raka brodawkowego wraz z ich wariantami oksyfilnymi, lecz w około 25% mylący obraz może wystąpić w przypadku guzków hiperplastycznych oraz przewlekłego limfocytowego zapalenia tarczycy [9, 10]. Większość guzów stanowią zmiany łagodne, choć w niektórych przypadkach ryzyko złośliwości sięga nawet 40% [11]. Brak precyzyjnej terminologii odzwierciedla ograniczenia BACC tarczycy w sytuacji, gdy rozpoznanie nowotworu złośliwego wymaga stwierdzenia naciekania torebki lub angioinwazji [11, 12]. Obraz cytologiczny kategorii „podejrzenie nowotworu pęcherzykowego” charakteryzuje bogato komórkowy aspirat zawierający jednolitą populację komórek pęcherzykowych,

minimalną ilość lub brak koloidu, układy przestrzenne o charakterze rozetek, a poszczególne komórki zawierają wydętne, zachodzące na siebie jądra, sporadycznie z obecnością atypii.

Diagnostyka nowotworów pęcherzykowych stanowi „szarą strefę” cytologii tarczycy. Trafność biopsji cienkoigłowej w rozpoznawaniu nowotworu złośliwego jest wysoka i zawiera się w przedziale 90–100%, natomiast w przypadku zmian pęcherzykowych średnio jedynie 20% z nich okazuje się zmianami złośliwymi w pooperacyjnym badaniu histopatologicznym [13, 14].

CEL PRACY

Celem pracy była wyodrębnienie czynników ryzyka raka tarczycy u chorych ze zmianami ogniskowymi lub guzami tarczycy zweryfikowanymi w BACC jako „podejrzenie nowotworu pęcherzykowego”. Identyfikacja klinicznych cech związanych z wyższym prawdopodobieństwem złośliwości w tej grupie pozwoliłaby wyodrębnić chorych, dla których obserwacja byłaby odpowiednim postępowaniem. Podejście zachowawcze w uzasadnionych przypadkach umożliwiłoby zmniejszenie chorobowości oraz ogólnych kosztów leczenia. Brak takich czynników może stanowić impuls do poszukiwania innych metod diagnostycznych, np.: biomarkerów w tkance guza bądź w osoczu, które podniosą zwiększyć prawdopodobieństwo klinicznie istotnego rezultatu.

MATERIAŁ I METODY

Do badania zakwalifikowano 110 kolejnych pacjentów operowanych w III Klinice Chirurgii Ogólnej UJCM w Krakowie na przełomie stycznia 2016 i marca 2020 z powodu wyniku BACC zweryfikowanej jako klasa IV według Bethesda (TBSRTC). Dane kliniczne zostały zebrane prospektywnie. Charakterystykę grupy pod względem cech demograficznych oraz klinicznych umieszczono w tabeli II. Kryteria włączenia stanowiły: potwierdzone cytologiczne rozpoznanie „podejrzenie nowotworu pęcherzykowego” lub „podejrzenie nowotworu z komórek Hürthle’a” u chorego dotychczas nieleczzonego z tego powodu, wiek od 18 do 90 r.ż., świadoma zgoda chorego na udział w badaniu. Kryterium wyłączenia stanowiła obecność cech nowotworu złośliwego, takich jak naciekanie okolicznych tkanek, podejrzenie przerzutów do węzłów chłonnych lub przerzutów odległych w badaniu klinicznym lub w przedoperacyjnych badaniach obrazowych.

Ocena zmienności badanych cech została przedstawiona poprzez średnie arytmetyczne, odchylenie standardowe (SD), 95% przedział ufności (95% CI) oraz odsetek częstości występowania (%). Opracowanie statystyczne wykonano w programie Statistica 13. Jednoczynnikową analizę statystyczną materiału przeprowadzono przy pomocy testów Chi² Pearsona oraz U Manna-Whitney’a. W ocenie istotności statystycznej przyjęto wartość $p < 0,05$. Opracowano ryzyko względne raka tarczycy dla poszczególnych parametrów.

Tabela I. Kategorie rozpoznania w badaniu cytologicznym tarczycy w klasyfikacji Betesda.

Kategoria rozpoznania	Ryzyko raka tarczycy
I – biopsja niediagnostyczna lub niesatysfakcjonująca	5–10% ^{b,c}
II – zmiana łagodna	<1% ^b (0–3%) ^c
III – zmiana pęcherzykowa bliżej nieokreślona (FLUS) lub atypia o nieokreślonym znaczeniu (AUS)	2,4–5,2% ^b (6–18%) ^c
IV – nowotwór pęcherzykowy lub podejrzenie nowotworu pęcherzykowego ^d	8,2–19% ^b (10–40%) ^c
V – podejrzenie złośliwości	75% ^b (45–60%) ^c
VI – nowotwór złośliwy	≥95–100% ^b (94–96%) ^c

^a na podstawie Bethesda System for Reporting Thyroid Cytopathology 2017

^b ryzyko raka przyjmowane w populacji polskiej, na podstawie: Diagnostyka i leczenie raka tarczycy – rekomendacje polskie. Endokrynol. Pol., 2018; 69: 34–74

^c ryzyko raka z pominięciem przypadków pooperacyjnych rozpoznania nieinwazyjnego nowotworu pęcherzykowego tarczycy o jądrach komórkowych przypominających raka brodawkowego (uznawanego obecnie za nowotwór niezłośliwy) – na podstawie: Cibas E.S., Ali S.Z.: The Bethesda system for reporting thyroid cytopathology. Thyroid 2017; 27: 1341–1346

^d W rozpoznaniu „podejrzenie nowotworu pęcherzykowego” mieści się także „podejrzenie nowotworu z komórek Hürthle’a” (dawniej „oksyfilnego”), które jest obciążone większym (15–25%) ryzykiem złośliwości i częściej stanowi jednoznaczne wskazanie do leczenia operacyjnego.

BAC – biopsja aspiracyjna cienkoigłowa

WYNIKI

W badaniu pooperacyjnym u 18 chorych rozpoznano raka tarczycy, najczęstszy podtyp stanowił RBT (66,6%), w połowie przypadków stwierdzono WPRBT (6/12). Spośród zmian łagodnych dominował gruczolak pęcherzykowy (49%). Raport histopatologiczny przedstawiono w tabeli III. Pacjentów podzielono na 2 grupy: ze zmianą łagodną (grupa 1) oraz z nowotworem złośliwym (grupa 2). Grupy poddano analizie pod względem: wieku, płci, obecności choroby Hashimoto, średnicy guza, odsetka rozpoznania cytologicznego „podejrzenie z komórek Hürthle’a” oraz sumarycznej liczby klinicznych i ultrasonograficznych cech zwiększonego ryzyka złośliwości według rekomendacji Polskiej Grupy Nowotworów Endokrynnych [15]. W grupie chorych ze zmianą złośliwą stwierdzono przewagę mężczyzn (16,6% vs. 13,1%, $p=0,31$), większy odsetek biopsji zweryfikowanych jako *podejrzenie nowotworu z komórek Hurthle’a* (55,6% vs. 45,7%, $p=0,075$), większy odsetek pacjentów z chorobą Hashimoto (33,3% vs. 31,5%, $p=0,55$), większy odsetek klinicznych i ultrasonograficznych cech zwiększonego ryzyka złośliwości (1,4 vs. 1,29, $p=0,71$), mniejszą średnicę guza (14,5 mm vs. 19,4 mm, $p=0,15$) oraz chorzy byli średnio o 2,3 roku młodsi (53,1 vs. 55,4 roku, $p=0,42$), lecz wyniki te nie były istotne statystycznie (Tab. IV). Ryzyko względne złośliwości związane z poszczególnymi cechami przedstawiono w tabeli V.

DYSKUSJA

Pomimo postępu diagnostyce, większej czułości badań obrazowych oraz możliwości wykorzystania technik immunohistochemicznych, w codziennej praktyce klinicznej wykluczenie nowotworu złośliwego w guzach pęcherzykowych na podstawie BACC jest niemożliwe. Rak tarczycy występuje średnio 3–4 razy częściej u kobiet, natomiast bardziej agresywny przebieg i większą śmier-

telność stwierdza się u mężczyzn. Postuluje się wpływ hormonów płciowych na patogenezę raka tarczycy, lecz dokładny mechanizm molekularny nie został poznany i na dzień dzisiejszy brakuje dowodów naukowych potwierdzających tę teorię. W kilku doniesieniach wskazano płeć męską jako czynnik ryzyka nowotworu złośliwego w przypadku chorych z nieokreśloną biopsją. Co więcej, wielośrodkowa analiza 21746 chorych operowanych w Europie i Stanach Zjednoczonych wykazała, że płeć męska wiąże się z istotnie wyższym ryzykiem złośliwości w każdej kategorii według systemu Bethesda (od I do VI) [16]. W analizowanej materiale dominowały kobiety w obu grupach, jednak odsetek mężczyzn w grupie z rozpoznaniem był większy [17–19].

Przewlekłe limfocytowe zapalenie tarczycy jest chorobą o podłożu autoimmunologicznym opisaną ponad 100 lat temu [20]. Na świecie ChH stwierdza się u około 1 na 1000 osób, najczęściej u kobiet w 4–6 dekadzie. [21]. Związek pomiędzy chorobą Hashimoto a nowotworem złośliwym tarczycy po raz pierwszy zaobserwowano w 1955 roku [22]. Częstsze występowanie RT u chorych z ChH zostało potwierdzone w licznych publikacjach [23–25], chociaż niektórzy autorzy nie wykazują takiej zależności [26]. W 2017 roku ukazały się 2 metaanalizy, które potwierdziły zwiększone ryzyko RBT u chorych z ChH. Na szczególną uwagę zasługuje praca Resende de Paiva i wsp. uwzględniająca wszystkie podtypy RT. Na podstawie 64,628 przypadków wykazali dodatnią korelację pomiędzy ChH a RBT, natomiast nie stwierdzono takiej zależności w przypadkach pozostałych podtypów RT [27, 28]. Spośród potencjalnych mechanizmów wymienia się uszkodzenia DNA w wyniku nadmiernego wytwarzania rodników tlenowych, jednak dokładny przebieg transformacji nowotworowej pozostaje nieznany [29]. Brakuje doniesień analizujących wpływ choroby Hashimoto na ryzyko raka wśród chorych z rozpoznaniem cytologicznym *podejrzenie nowotworu pęcherzykowego*. Zależność

Tabela II. Charakterystyka grupy badanej

	Wartość średnia [min – max]	Odchylenie standardowe [SD]	Liczba chorych [n,%]
Wiek [lata]	55,1 [19-88]	18,14	
Rozmiar guza [mm]	18,7 [3-80]	15,85	
Czynniki ryzyka złośliwości	1,32 [0-4]	0,82	
Choroba Hashimoto			35 (31,8%)
Rozpoznanie cytologiczne – podejrzenie nowotworu pęcherzykowego/ podejrzenie nowotworu z komórek oksyfilnych			58/52 (52,7%)
Płeć (M/K)			15/95 (13,6%)

Czynniki ryzyka złośliwości [15]:

- kliniczne

- a) przerzuty do węzłów chłonnych lub przerzuty odległe;
- b) ekspozycja szyi na promieniowanie jonizujące w wywiadzie;
- c) szybki wzrost guzka;
- d) guzek twardy, zrośnięty z otoczeniem;
- e) średnica guzka > 4 cm;
- f) pojawienie się guzka tarczycy przed 20. rokiem życia lub po 60. roku życia;
- g) porażenie nerwów krtaniowych, szczególnie jednostronne.

- ultrasonograficzne

- a) cechy wskazujące na możliwość przerzutu raka tarczycy do węzłów chłonnych szyjnych;
- b) cechy naciekania torebki tarczycy wraz z naciekaniem okolicznych narządów lub bez;
- c) obecność mikrozwapnień w zmianie ogniskowej tarczycy;
- d) charakter lity i hipoechogeniczność zmiany ogniskowej;
- e) kształt zmiany ogniskowej („wysokość > szerokość”);
- f) nieregularne granice zmiany;
- g) cechy wzmożonego, chaotycznego przepływu naczyniowego centralnie w zmianie.

ta wydaje się być wartą zbadania ze względu na obecność komórek oksyfilnych w przebiegu ChH, co może prowadzić do błędnej diagnozy cytologicznej i pochopnej kwalifikacji do leczenia operacyjnego. W badanym materiale we wszystkich przypadkach współistnienia choroby Hashimoto i raka tarczycy (6/18) rozpoznano podtyp brodawkowaty, w 3 przypadkach WPRBT. Przewaga pacjentów z ChH w grupie chorych z rakiem tarczycy nie była istotna statystycznie.

Pojawienie się zmiany ogniskowej w tarczycy przed 20 rż oraz po 60 rż wiąże się z jej większym ryzykiem złośliwości [30]. W przypadku dzieci i młodych dorosłych nabłonek tarczycy wykazuje wyższy stopień proliferacji i zwiększoną podatność na czynniki zewnętrzne, np: promieniowanie jonizujące oraz częstsze występowanie niektórych mutacji prowadzących do raka tarczycy, jak rearanżacja RET/PTC [31]. Niedoczynności tarczycy oraz osłabiona funkcja układu odpornościowego są najprawdopodobniej odpowiedzialne za większą częstość raka tarczycy w grupie osób po 60 rż [32]. W naszym materiale średni wiek chorych w grupie 1 wyniósł 55,4 roku, natomiast w grupie 2 – 53,1 roku. Różnica nie była istotna statystycznie ($p=0,58$).

Rozmiar guza ma istotny wpływ na ryzyko rozsiewu, nawrotu choroby oraz rokowanie. Według polskich zaleceń guz o średnicy >4 cm stanowi wskazanie do zabiegu operacyjnego w przypadku biopsji nieokreślonej. W analizowanym materiale w 1 przypadku guz o średnicy >4 cm okazał się mieć złośliwy charakter. Ponadto, w grupie chorych ze zmianami łagodnymi średni rozmiar guza okazał się być

wiekszy o 4,9 mm (19,4 mm vs. 14,5 mm), lecz wynik nie jest statystycznie istotny ($p=0,15$) [15, 33].

Wytyczne ATA oraz rekomendacje Polskiej Grupy Nowotworów Endokrynnych wyróżniają kliniczne i ultrasonograficzne cechy zwiększonego ryzyka złośliwości zmian ogniskowych tarczycy [15]. W przeprowadzonym badaniu każdej spośród wyżej wymienionych cech przypisano 1 punkt, po czym zsumowano je i porównano średnią wartość punktową w obu grupach. Średnio więcej cech zwiększonego ryzyka złośliwości stwierdzono w grupie 2 (1,4 vs. 1,29), przy czym nie uzyskano istotności statystycznej ($p=0,65$).

Obecność komórek oksyfilnych w bioptowanych zmianach ogniskowych tarczycy stanowi powszechnie uznany czynnik ryzyka nowotworu złośliwego [15,35]. W przypadku populacji polskiej co najmniej 15% takich guzów okazuje się być RT, natomiast w populacji ogólnej odsetek ten sięga nawet 40%. Chorzy z takim rozpoznaniem cytologicznym powinni być kwalifikowani do leczenia operacyjnego. U chorych z wynikiem BACC *podejrzenie nowotworu z komórek Hurthle'a* stwierdzono częstsze występowanie raka tarczycy, lecz nie wykazano istotności statystycznej (55,6% vs 45,7%, $p=0,075$).

Z uwagi na brak możliwości przedoperacyjnego odróżnienia raka od gruczolaka pęcherzykowego tarczycy wielu autorów poszukiwało klinicznych cech związanych z wyższym ryzykiem złośliwości w tej grupie chorych. Niektóre doniesienia sugerują stratyfikację nowotworów pęcherzykowych wyróżniając zmiany wysokiego ryzyka, które

Tabela III. Wynik badania histopatologicznego .

	Grupa 1 N=92	Grupa 2 N=18
Wynik badania histopatologicznego	Gruczolak pęcherzykowy = 45	Rak brodawkowaty, wariant klasyczny = 6, wariant pęcherzykowy = 6
	Wole guzkowe = 36	Rak pęcherzykowy = 5
	Przewlekłe zapalenie = 11	Rak niskozróżnicowany = 1

Tabela IV. Czynniki ryzyka raka tarczycy

	Grupa 1	Grupa 2	p
Płeć (M/K)	12/80	3/15	0,31
Średni wiek [lata]	55,4 (±19,1)	53,1 (±17,6)	0,58
Średnia liczba czynników ryzyka złośliwości klinicznych i USG	1,29 (± 0,91)	1,4 (± 1,03)	0,65
Choroba Hashimoto [n (%)]	29/92 (31,5)	6/18 (33,3)	0,55
Rozpoznanie cytologiczne – pęcherzykowy/ Onkocytny [n,%]	50/42 (54,3)	8/10 (44,4)	0,075
Średnica guza [mm]	19,4 (±11,2)	14,5 (± 9,7)	0,15
Razem	N= 92	N= 18	

Tabela V. Ryzyko względne raka tarczycy.

Cecha	Liczba chorych	Ryzyko złośliwości (n = 18)	
		Przedział ufności (95% CI)	P
Płeć			
Mężczyzna	15	0,9 (0,85 – 1,71)	0,31
Kobieta	95	1,00	
Wiek			
Ponad 55 lat	51	0,76 (0,64 – 1,59)	0,61
Poniżej 55 lat	69	1,00	
Choroba Hashimoto			
Tak	35	0,94 (0,91 – 1,18)	0,55
Nie	75	1,00	
Średnica guza			
Ponad 4 cm	12	1,34 (0,83 – 1,67)	0,47
Poniżej 4cm	98	1,00	
Podejrzenie npl z komórek Hürthle’a			
Tak	52	1,05 (0,89 – 1,21)	0,075
Nie	58	1,00	
Suma klinicznych i ultrasonograficznych cech ryzyka złośliwości			
1 lub więcej	87	1,46 (0,89 – 1,74)	0,23
0	23	1,00	

należy poddać operacji oraz niskiego ryzyka złośliwości, dla których obserwacja będzie właściwym postępowaniem [15]. Istotne znaczenie ma średnica zmiany. RT występuje częściej w guzach pęcherzykowych przekraczających >3–4 cm w największym wymiarze. Baloch i wsp., oprócz średnicy, do czynników ryzyka, zaliczyli płeć męską oraz wiek >40 lat, z kolei Davis i wsp., dodatkowo wykazali wpływ radioterapii okolicy głowy i szyi na występowanie RP [13,34].

Średnica guza odgrywa istotną rolę również w przypadku podejrzenia nowotworu z komórek Hurthle’a. Według

pracy Bronner i LiVolsi zmiany przekraczające 4 cm w 80% mają charakter złośliwy [35].

W celu stratyfikacji ryzyka RT można wykorzystać badania obrazowe. Wytyczne ATA z 2015 roku podkreślają wysoką wartość USG tarczycy w ocenie zmian o nieokreślonym potencjale złośliwości [36]. W zależności od doniesień do cech zwiększających ryzyko złośliwości zalicza się: mikrozwapnienia, kształt zmiany “wyższy niż szerszy”, nieregularny brzeg, lity charakter, brak halo oraz hypoechogeniczność [37]. Na podstawie metaanalizy 9 badań obserwacyjnych Remonti i wsp. wykazali, że nie ma pojedynczej cechy w USG,

która w przypadku nieokreślonej biopsji charakteryzowałaby się akceptowalną dokładnością [38].

Liczne doniesienia wskazują na potrzebę wprowadzenia kryteriów ultrasonograficznych u chorych z nieokreśloną BACC, które pozwoliłyby na stratyfikację w obrębie tej grupy [39, 40]. Amerykańskie Towarzystwo Radiologiczne zaproponowało system punktowy oparty na 5 cechach. Zmiany ogniskowe zostają zaklasyfikowane do odpowiedniej kategorii na podstawie liczby punktów otrzymywanych za określone cechy: TR2 (2 pkt); TR3 (3 pkt); TR4 (4–6 pkt, TR4a – 4 pkt, TR4b – 5 pkt, TR4c – 6 pkt) i TR5 (≥ 7 pkt) [41, 42]. Oba modele (według ATA oraz ACR) zostały porównane w pracach Middletona i wsp. oraz Barbosa i wsp. Autorzy konkludują, że zmiany ogniskowe zweryfikowane jako podejrzenie nowotworu pęcherzykowego z wysokim ryzykiem złośliwości na podstawie kryteriów ATA 2015 lub wynikiem 4b/4c/5 według ACR TI-RADS wiążą się z gorszym rokowaniem i stanowią wskazanie do leczenia operacyjnego. W przypadku mniejszego ryzyka złośliwości można rozważyć badania molekularne (jednak metody te są drogie i w Polsce nierefundowane) [41, 42].

Postępy w dziedzinie biologii molekularnej zostały wykorzystane w diagnostyce nowotworów pęcherzykowych tarczycy. Badania molekularne pozwalają na rozróżnienie łagodnych i złośliwych guzów, zwłaszcza w sytuacji niejednoznacznego wyniku badania cytologicznego (według klasyfikacji Bethesda kategoria III–V) poprzez wykrywanie mutacji typowych dla RT [43, 44]. Wyróżnia się 2 ścieżki diagnostyczne, które wynikają z charakterystyki stosowanych testów. W przypadku niewielkiego ryzyka raka stosuje się testy wykluczające. Zalicza się do nich *Afirma Gene Expression Classifier* (GEC), który dostarcza firma Veracyte. Najnowsza wersja testu charakteryzuje się czułością 91,1% oraz specyficznością 68,3% [44]. Jeżeli ryzyko raka jest wysokie, należy wykonać test potwierdzający, np. ThyroSeq V3 Genomic Classifier, który charakteryzuje się czułością 94%, specyficznością 82%, wartością predykcyjną ujemną 97% i wartością predykcyjną dodatnią 66%. Zastosowanie testu ThySeq V3 pozwala uniknąć diagnostycznego zabiegu operacyjnego u ponad 60% [45].

Doniesienia z ostatnich lat pokazują, że diagnostyka zmian pęcherzykowych tarczycy jest tematem otwartym. Pomimo postępu w dziedzinie badań obrazowych, biologii molekularnej oraz immunohistochemii, znaczny odsetek chorych wymaga zabiegu operacyjnego w celu wykluczenia nowotworu złośliwego. Wobec inwazyjności, potencjalnych powikłań oraz wysokich kosztów diagnostyczny zabieg operacyjny nie jest optymalnym rozwiązaniem, jednak na dzień dzisiejszy pozostaje postępowaniem z wyboru. Wymienione niedogodności mogłyby zostać wyeliminowane dzięki badaniom molekularnym. Koszty, jakie trzeba ponieść są niższe, niż w przypadku zabiegu operacyjnego, natomiast mniej opłacalne jest wykonywanie ich u każdego chorego z rozpoznaniem cytologicznym Bethesda IV. Kluczowym wydaje się wyselekcjonowanie cech klinicznych i ultrasonograficznych, w przypadku których testy molekularne będą miały najwyższą wartość diagnostyczną i opłacalność.

W badaniu Autorów nie wyselekcjonowano czynników ryzyka, które wiązałyby się z niskim ryzykiem nowotworu złośliwego u chorych z wynikiem BACC *podejrzenie nowo-*

tworu pęcherzykowego. Z jednej strony ograniczenie badania stanowi niewielka liczebność analizowanej grupy. Z drugiej strony należy podkreślić trudności w pozyskiwaniu materiału związane z charakterem zmian. Szerokie spektrum rozpoznań histopatologicznych odpowiadających kategorii IV według klasyfikacji Bethesda czyni tę grupę niejednorodną, co utrudnia wyszukanie czynników ryzyka różnicujących np.: raka i gruczolaka pęcherzykowego. Konieczne jest przeprowadzenie dalszych badań uwzględniających większe grupy chorych, zwłaszcza do czasu powszechnego wprowadzenia badań molekularnych lub innych testów o wysokiej trafności diagnostycznej

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ORIGINAL ARTICLE

INFLUENCE OF TWO-HOUR TOURNIQUETS ISCHEMIA OF LIMB AND ACUTE BLOOD LOSS ON SYSTEMIC DISORDERS OF THE BODY IN THE REPERFUSION PERIOD (EXPERIMENTAL STUDY)

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ABSTRACT

The aim: to find out the effect of a two-hour tourniquets ischemia of the limb and acute blood loss on systemic disorders of the body in the postperfusion period.

Materials and methods: The experiments were performed on 96 nonlinear male rats weighing 200–220 g. All experiments were performed under sodium thiopental anesthesia. In the first experimental group, two-hour tourniquets limb ischemia was simulated. In the second experimental group, acute blood loss was modeled. In the third experimental group, these lesions were combined. In 1 and 2 hours, as well as in 1, 7 and 14 days, the biliary function of the liver was determined in the experimental animals.

Results: Two-hour tourniquets ischemia of the limb in the reperfusion period compared with the control was accompanied by a significant decrease in the rate of bile excretion, which reached a minimum value in 3 h – 1 day of the experiment and returned to the control level in 14 days. After the simulation of acute blood loss, the rate disturbance of bile excretion became significantly greater in all observation periods. The complication of acute ischemia-limb reperfusion caused a greater decrease in the rate of bile excretion with a maximum in 1 day of the experiment. Under these conditions, at 1, 7, and 14 days, the indicator was statistically significantly lower than in the other study groups.

Conclusions: In the case of acute blood loss, complicated by two-hour tourniquets ischemia of the limb, the reperfusion period is accompanied by a summation of the negative impact of blood loss, ischemia and reperfusion of the limb on the functional state of the liver, which is a significant decrease in the rate of bile excretion with a maximum in 1 day, which further increases, but up to 14 days does not reach the level of control.

KEY WORDS: blood loss, tourniquets, ischemia, reperfusion, bile excretion

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INTRODUCTION

Today one of the current problems is the increasing incidence of terrorist attacks and local armed conflicts, which has an impact on public health, [1]. Under these circumstances, the main reason of the death of victims and injured is acute blood loss [2]. An effective tool to stop massive external limb bleeding is considered to be a tourniquet that completely stops arterial blood flow for a safe period of up to two hours [3].

It is established that two hours of full bleeding stop in the limb causes damage to the nerves and vessels under the tourniquet, which leads to myonecrosis, rhabdomyolysis, deep vein thrombosis [4]. Some authors have shown that even greater disturbances in soft tissues of the limb occur after its reperfusion [5]. The processes of lipid peroxidation are significantly activated, the destruction of cell membranes is increased in muscle tissue, which causes inflammation of ischemia-induced local areas. The question of the influence of inflammatory mediators and toxic metabolites which enter the systemic circulation from the ischemic limb on vital organs remains debatable.

THE AIM

To find out the effect of two-hour tourniquets ischemia of the limb and acute blood loss on systemic disorders of

the body in the reperfusion period (due to model of liver biliary function).

MATERIALS AND METHODS

The experiments were performed on 96 non-linear male rats weighing 200–220 g according to the rules of the “European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes” (European Convention, 1984). Bioethics Commission of I. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine found conducted experiments to be in compliance with the basic ethical standards of work with laboratory animals (No. 54 dated 27.08.2019).

All animals were divided into five groups: one control and four experimental (6 rats in each group). The first experimental group included animals that modeled ischemia-reperfusion of the limb. Under sodium thiopental anesthesia (40 mg•kg⁻¹ of animal weight) animals were put proximally superimposed elastic strip tourniquet “SWAT-T” (USA) on the left leg, width of it is 10 mm, duration up to 120 minutes. The tourniquet was tightened according to the effective pressure indicator on it which stops the blood flow. The anemia of the limb was further confirmed by the rheographic method. In the second ex-

Table I. Dynamics of biliary velocity (ml·H⁻¹·kg⁻¹) after acute blood loss complicated by ischemia-reperfusion of the limb (Me (LQ; UQ)) - median (lower and upper quartile)

Experimental group	Reperfusion period				
	2 h	3 h	1 day	7 day	14 day
	Control = 2.25 (2.19; 2.32) (n=6)				
The first experimental group Ischemia-reperfusion	2.15 (2.06; 2.19) (n=6)	1.74 ^{*2h} (1.72; 1.76) (n=6)	1.83 ^{*2h} (1.81; 1.93) (n=6)	2.09 ^{*3h,1d} (2.06; 2.16) (n=6)	2.32 ^{2h,3h,1d,7d} (2.24; 2.47) (n=6)
The second experimental group Blood loss	1.98 [*] (1.85; 2.07) (n=6)	2.45 ^{*2h} (2.34; 2.56) (n=6)	1.58 ^{*2h,3h} (1.46; 1.64) (n=6)	1.83 ^{*3h,1d} (1.74; 1.93) (n=6)	1.95 ^{*3h,1d} (1.90; 2.03) (n=6)
The third experimental group Ischemia-reperfusion + Blood loss	1.84 [*] (1.82; 1.96) (n=6)	1.72 [*] (1.67; 1.83) (n=6)	1.29 ^{*2h,3h} (1.19; 1.38) (n=6)	1.58 ^{*2h,1d} (1.50; 1.69) (n=6)	1.63 ^{*2h,1d} (1.56; 1.77) (n=6)
p ₁₋₂	>0.05	<0.05	<0.05	<0.05	<0.05
p ₁₋₃	<0.05	>0.05	<0.05	<0.05	<0.05
p ₂₋₃	>0.05	<0.05	<0.05	<0.05	<0.05

Notes:

- * – differences in control group are statistically probable (p<0.05);
- p₁₋₂ – probable differences between the experimental groups 1 and 2;
- p₁₋₃ – probable differences between the experimental groups 1 and 3;
- p₂₋₃ – probable differences between experimental groups 2 and 3;
- ^{3h,4h,1d,7d} – differences due to third and fourth h, respectively, as well as on 1 and 7 day of the experiment are statistically probable (p<0.05).

perimental group under conditions of sodium thiopental anesthesia, acute blood loss was simulated by incision of the femoral vein. After substantiation of blood volume loss up to 20 % of circulating blood volume, the animals were provided with hemostasis. In the third experimental group, these injuries were combined.

In the control group, the animals were anesthetized using an equivalent dose of sodium thiopental, a tourniquet was applied for 2 h without stopping the blood flow, and subsequently taken for studies in 1 h.

In 1 and 2 hours, as well as in 1, 7 and 14 days functional state of the liver was determined in experimental animals. The biliary function of the liver was studied in each experimental group by catheterization of the common bile duct and bile intake during 1 h under conditions of sodium thiopental anesthesia (60 mg kg⁻¹ mass) [6]. The rate of bile excretion was calculated based on the hour and kilogram of animal's body weight. After bile collection, rats were taken out from the experiment by total bloodletting from the heart, which in the early period of the experiment corresponded to 2 and 3 h of reperfusion.

The estimation of probable differences between the experimental groups was performed using the non-parametric Mann-Whitney criterion.

RESULTS

Studies have shown (Table I) that in experimental group 1, in which the ischemia-reperfusion of the limb was simulated, in 1 h of reperfusion, the rate of bile excretion did not change significantly compared to the control group. However, in 3 h of the reperfusion period, the index de-

creased by 22.7 % compared to the control (p<0.05) and by 19.1 % compared to 2 h of observation (p<0.05) and remained at the same level up to 1 day of experiment. In 7 days, the rate of bile excretion increased and was statistically significantly higher than in the previous observation period (p<0.05), but remained significantly lower than in the control (p<0.05). In 14 days, the index increased further and reached the level of the control group (p<0.05).

In the case of acute blood loss (experimental group 2), the rate of bile excretion was significantly lower than in the control in 2 h of reperfusion (by 12.0 %, p<0.05). In 3 hours, the indicator became even smaller: by 32.9 % compared to the control (p<0.05) and by 23.3% compared to the previous observation period (p<0.05). In 1 day, the rate of bile excretion remained at the level of 3 h (p<0.05) and was 29.8 % lower than in the control. In the future, the indicator increased, but by the 14th day it did not reach the level of control and was 13.3 % lower (p<0.05).

In the case of combination of acute blood loss with limb ischemia-reperfusion (experimental group 3), the rate of bile excretion in 2 h was 18.2 % lower than in the control (p<0.05). In 3 h of the conducting experiment, the index decreased even more – by 20.6 % compared to the previous observation period (p<0.05) and by 35.1 % – compared to the control (p<0.05). In 1 day the indicator reached the minimum value. It decreased by 42.7 % (p<0.05) compared to the control and was statistically significantly smaller than in the previous observation periods (p<0.05). Further up to 7 days the indicator increased (by 22.5% compared to the previous observation period, p<0.05) and remained at the same level up to 14 days.

Comparison of the experimental groups with each other showed that in 2 h of the experiment in the experimental group 3 the rate of bile excretion was significantly lower than in the experimental group 1 (by 14.4 %, $p_{2-3} < 0.05$). In 3 h, the indicator was statistically significantly lower in experimental groups 2 and 3 compared to experimental group 1 (13.2 %, $p_{1-2} < 0.05$ and 16.1 %, $p_{1-3} < 0.05$, respectively). In 1, 7 and 14 days of the experiment, the indicator in experimental group 3 was statistically significantly lower than in experimental groups 1 and 2 ($p_{1-3} < 0.05$, $p_{2-3} < 0.05$) and significantly lower in experimental group 2 compared with experimental group 1 ($p_{1-2} < 0.05$).

DISCUSSION

The obtained results indicate that in the case of reperfusion of the limb in two hours of ischemia, preconditions arise that adversely affect the functional state of the liver, which is manifested by a decrease in the rate of bile secretion with a minimum of 3 h and 1 day of experiment and normalization to 14 days. On the base of the pathogenesis of biliary tract disorders is a decrease in the formation of bile components, slowing of the excretion process in the biliary tract, swelling of the liver parenchyma with impaired bile flow, and impaired bile duct motility [7]. Given the membrane dependence of bile formation and excretion processes, it can be assumed that toxins from the ischemic limb entering the bloodstream will cause damage to cellular and subcellular membranes, primarily by enhancing the processes of lipid peroxidation, which, in turn, work separately [8]. Under these conditions, the source of systemic prooxidant action is muscles, vessels, and nerve fibers that have been ischemic for two hours and are subject to reperfusion damage [9].

In conditions of acute blood loss, bile ductility disorders were observed during all observation periods, which was higher than in experimental group 1. By 14 days the index did not reach the level of the control group. The decrease in bile excretion is obviously due to the development of hypovolemic shock, which is characterized by "centralization" of blood circulation and a decrease in the blood supply to the organs of the gastrointestinal tract [10].

In conditions of acute blood loss, complicated by two-hour ischemia-reperfusion of the limb, the disorders were more pronounced. If in 2 and 3 h of experiment in this group the rate of bile excretion was at the level of animals with acute blood loss, then in the future the indicator became statistically significantly lower. It can be assumed that in experimental group 3 there is an over-placement of pathogenic mechanisms of limb ischemia-reperfusion and acute blood loss. Organ specificity of biliary function puts it in a number of important and informative criteria and allows to monitor the status of hepatocyte microsomes, where cholesterol synthesis of bile acids and conjugation of bilirubin with glucuronic acid occur; the state of cytoplasmic membranes of the sinusoidal pole of hepatocytes that capture bilirubin and cholesterol from the blood; the state of the hepatocytes biliary pole, where the compo-

nents of bile are transferred to the primary bile capillary; the state of the bile ducts, which provide the outflow of bile into the duodenum [7]. In addition to, a number of publications investigating biliary liver function have been successfully used as a model of organ dysfunction and insufficiency [11].

The result we have obtained is of considerable practical importance, since in the course of assisting the victims and the injured with acute blood loss, it aims at obligatory consideration of the fact of ischemia and reperfusion of the limb, which can significantly deepen the course of hypovolemic shock and stimulate the development of organ dysfunction. All this requires finding effective means of eliminating the effects of limb ischemia-reperfusion in the case of acute blood loss.

CONCLUSIONS

1. Two-hour ischemia of the limb in the reperfusion period is accompanied by a significant decrease in the rate of bile excretion, which reaches a minimum value in 3 h – 1 day of the experiment and returns to the control level in 14 days.
2. After modeling of acute blood loss, the amplitude of bile duct velocity disorders in all observation periods becomes statistically significantly greater than in the case of ischemia-reperfusion, but their dynamics remains similar with a maximum decrease in 3 h – 1 day and an increase – in 14 days, which does not reach level of control.
3. The complication of acute blood loss of limb ischemia-reperfusion is accompanied by a summation of their negative effect on the functional state of the liver with a maximum in 1 day of the experiment. Under these conditions, in 1, 7 and 14 days, the rate of bile excretion is statistically significantly lower than in the experimental group in which the most acute blood loss was simulated.

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ORIGINAL ARTICLE

MATERNAL PSYCHOSOCIAL STRESS AND LABOR DYSTOCIA

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ABSTRACT

The aim: to study the possible role of psychosocial stressors in the emergence of anomalies of childbirth, as well as to examine their nature using the example of pregnant women who have been forcefully displaced from Donetsk and Luhansk regions of Ukraine.

Materials and methods: 115 internally displaced pregnant women from Donetsk and Luhansk regions, were surveyed. All women underwent a comprehensive neuropsychological examination of their psycho-emotional status via interviews, questioning, and psychological testing (Spielberg-Haning's Situational and Personal Anxiety Scale). The following analysis of the childbirth process' nature was conducted.

Results: The prevalence of high level of situational and personal anxiety by the Spielberg-Haning's test were determined in the internally displaced women. This indicates a high level of social anxiety (stress) in these pregnant women.

Labours in women in this group can be characterized by 3 main features: rapid (precipitated) labour with high levels of obstetric traumatism (tear of perineum) and labour medicalization (EDA). The characteristics described above are due to such labour activity anomalies as hypertensive uterine dysfunction – the cases when the speed and strength of uterine contractions significantly exceed the normative parameters.

The reasons for the high level of pain in labour in displaced women could be objective and subjective factors: excessive nociceptor irritation due to cervical-uterine dysfunction and impaired individual perception of pain.

Conclusions: Chronic psychosocial stress has an impact on labour activity in displaced women. A mandatory standard for managing such pregnancy is psychological counselling and correction of the identified disorders.

KEY WORDS: stress, anxiety, internally displaced women, labor anomalies

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INTRODUCTION

Childbirth is always associated with a physiological process that is absolutely natural for every woman, and ends with giving birth to a healthy baby. However, the anomalies of a childbirth process as a clinical and theoretical problem have not lost their relevance up to date, and still accompany 11-42% of all labours [1].

The consequences of these are an increase infectious complications risk, maternal injuries, including tear of the uterus and the perineum with subsequent development of pelvic floor muscle prolapse, bleeding, neonatal acidosis and injuries of the neonates. All around the globe the anomalies of childbirth are holding the first place among the indications for the first caesarean section, and that leads to a regular increase in the frequency of anomalies in the future. Therefore, it becomes not only a medical problem but also a social one. The negative experience of pathological labour subsequently causes deterioration of the quality of life of young women, disruption of relationships with their dearest environment; it causes conflicts in the family and arises fears for the subsequent pregnancies and childbirth, as well as leads to the complication during next pregnancy and labour. The "vicious circle" of new unresolved problems comes up again.

Mechanisms of inception and progression of child delivery include many complex interrelated psycho-neu-

roendocrine processes. The prerequisites for a normal childbirth are the smooth operation of this entire system. Any violation of any link leads to complications in labour. But despite the fact that neuroendocrine regulation of contractile activity is already well studied, its psychological aspects are either debatable or simply out of the sight of researchers and clinical physicians. One of the factors that may have a serious impact on childbirth process is a stress.

Every pregnant woman is typically stressed about the changes her body undergoes in relation to pregnancy, as well as about well-being and condition of the baby inside her, upcoming labour and motherhood. These refer to the physiological pregnancy stress, which in moderate manifestations is considered to be normative. The effects of additional stressors, such as the so-called psychosocial stressors that include changes in personal life, loss of status, work, house, financial stress, etcetera, lead to additional mobilization of the body's defences and changes in the pregnant woman's behaviour in order to be able to overcome them[2]. Altogether these factors create preconditions for the complicated course of pregnancy and childbirth. Therefore, in order to improve the quality of obstetric care, its adjustment to the needs of different social groups, there is a need for a more detailed study of this issue.

THE AIM

To study the possible role of psychosocial stressors in the emergence of anomalies of childbirth, as well as to examine their nature using the example of pregnant women who have been forcefully displaced from Donetsk and Luhansk regions of Ukraine.

MATERIALS AND METHODS

In order to achieve this goal, we surveyed 115 internally displaced pregnant women from Donetsk and Luhansk regions, who were monitored and then gave birth in the Kyiv Municipal Maternity Clinic №2. All patients were examined in accordance with the current regulations in obstetrics and gynaecology.

Inclusion criteria: single pregnancy, 37-41 weeks of gestation, absence of severe extra-genital pathology, spontaneous onset of labour.

Exclusion criteria: pregnancy as the result of assistive reproductive technologies, multiple pregnancy, <37 and > 41 weeks of gestation, presence of severe extra-genital pathology, induction of labour, planned (elective) or urgent surgical delivery.

All women underwent a comprehensive neuropsychological examination of their psycho-emotional status via interviews, questioning, and psychological testing (Spielberg-Hanin's Situational and Personal Anxiety Scale). The analysis of the childbirth process' nature was conducted according to the clinical observation data, partograms and instrumental methods of research (tocography).

We formed a group of 70 women with a physiological pregnancy for the comparison purposes.

RESULTS

According to the analysis of the anamnestic data (Table 1), both groups had no significant difference as per main indicators ($p > 0.05$). In the first group ($p > 0.05$) somewhat older women pregnant with the first child, mainly with higher education and married prevailed. Significant difference between the groups was determined by social and living conditions: the women of group 1 were characterized by a high percentage of the unsettled mode of life and unsatisfactory financial situation, which became one of the causes of the corresponding psychological problems. According to several recent Cochrane reviews, these factors play important role in preventing gestational complications. The need for the correction of these factors refers to the so-called psychosocial interventions that should be addressed during pregnancy. Psychosocial intervention is "any non-pharmacological intervention conducted in a therapeutic context at the individual, family or group level" (WHO 2009) and is "focused on psychological and / or social factors rather than biological ones", and directly or indirectly changes woman's behaviour, cognition and / or emotional state [3,4].

We are not capable of preventing the effects of global social factors on pregnancy and childbirth, yet we consider

encouraging a partner support as an available and cost-effective method of assisting and behavioural correction during childbirth to be the golden standard of obstetric care. Regrettably, according to the obtained data, in the group of internally displaced women the partner was present during the childbirth only in every second case (versus 84.3% in the comparison group). Special attention should be paid to this fact in course of dealing with this category of patients.

The methodology for stress research in pregnant women is a highly debated issue worldwide. For example, the conventional definition of the stress hormone, e.g. cortisol, has certain features during gestation: a pregnant woman's reaction to it is not always dependent on its level; its high concentrations may be a normal value for a certain woman, and the foetus is protected from its influence by a specific placental enzyme – 11- β -hydroxysteroid dehydrogenase. Therefore, a psychological testing is most commonly used with the help of Spielberg-Hanin's Situational and Personal Anxiety Scale. The level of anxiety can be considered as the level and type of stress that is important for the occurrence of pathology. The level of situational (reactive) anxiety (level of anxiety at the moment) and personal anxiety (anxiety as a stable personal characteristic) are determined. Anxiety is an acquired behavioural dysfunction that causes an individual to perceive a wide range of objectively safe events as threats, responding to them by falling into a state of anxiety [5].

Every person individually perceives and responds to the same situation as to a stressful or a non-stressful one.

Table 1. Sociodemographic characteristics of the examined groups.

	1 group, n=115	2 group, n=70
Age, years	29,08 $\pm$ 5,15	26,5 $\pm$ 3,6
Nulliparous	67(58,3%)	46(65,7%)
Multiparous, first labor	29(25,2%)	15(21,4%)
Multiparous,	19(16,5%)	9(12,9%)
Higher education	89(77,4%)	58(82,8%)
Secondary education, incomplete higher education	26(22,6%)	12(17,2%)
Registered marriage	96(83,5%)	62(88,6%)
Unregistered marriage	19(16,5%)	8(11,4%)
Partner at birth	61(53,0%)	59(84,3%)
Satisfactory financial situation	80(69,6%)	57(81,4%)
Unsatisfactory financial situation	35(30,4%)	13(18,6%)
Satisfactory living conditions (own apartments)	12(11,4%)	42(60%)
Satisfactory living conditions (rent)	90(78,3%)	20(28,6%)
Unsatisfactory living conditions	13(11,3%)	8(11,4%)

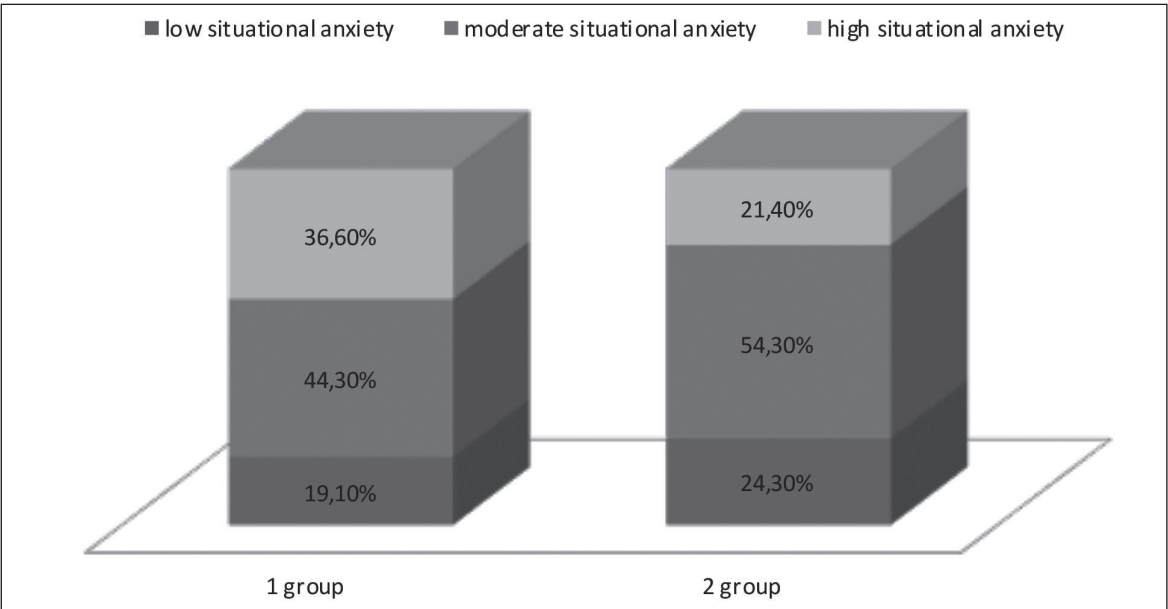


Fig. 1. Personal Anxiety Level of the examined groups

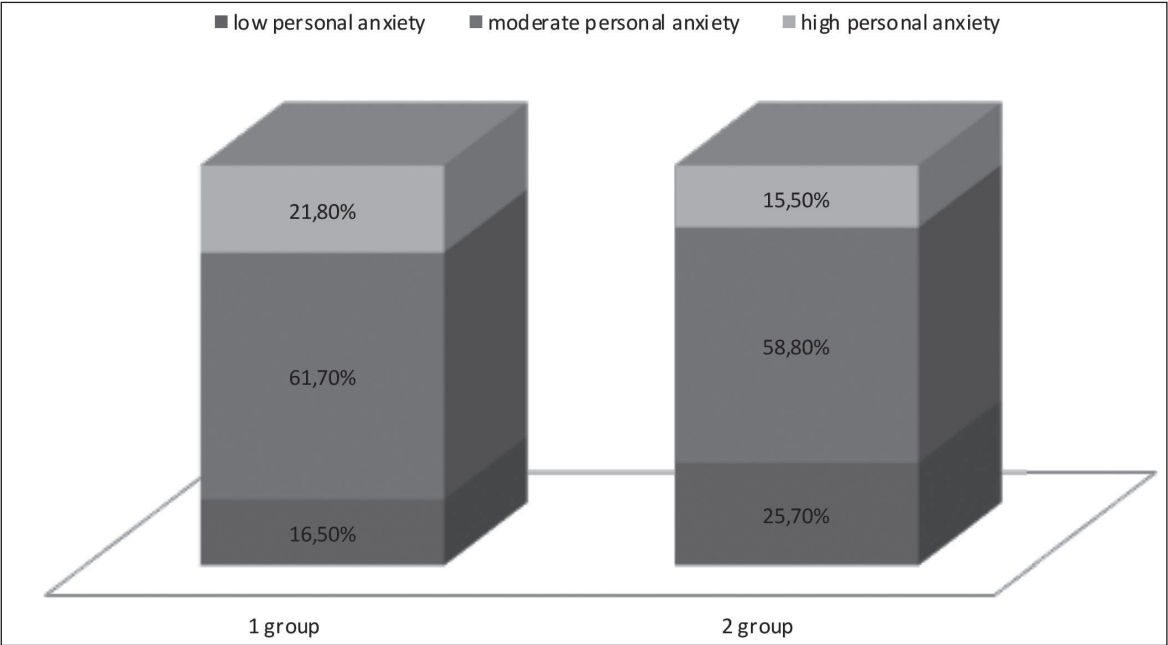


Fig. 2. Situational Anxiety Level of the examined groups

A moderate level of anxiety is a normative value for humans because it allows them to adapt to the surrounding changes. However, its severity can be detrimental, not always in line with the magnitude of the objective danger, and thus can further be transformed into a personal anxiety.

Situational (reactive) anxiety more closely reflects an individual's response to a certain threatening situation, and it is associated with social challenges in particular. It is caused by emotional experiences related to a state of uncertainty, instability, as well as nebulousness of the future, sensation of lack of support from the loved ones, etcetera. Reactive anxiety is characterized by tension, emotional anxiety, nervousness and behavioural changes. It affects the process of psychological adaptation of a pregnant woman to the very fact of pregnancy, future childbirth and the

postpartum period, as well as the formation of maternal behaviour. This psychological testing does not clearly identify a particular stressor as a cause for the anxiety of patients, but it allows identifying risk groups for them to take appropriate preventive measures.

When analysing the results of the Spielberg-Haning's test, the prevalence of middle and high levels of anxiety is determined in pregnant women of both groups, especially in the group of the internally displaced women (fig.1,2). This indicates a high level of stress in these pregnant women.

Moreover, such an unfavourable structure of indicators is common for both situational and personal anxiety. Thus, it cannot be explained by just sole fact of the upcoming really stressful event – that is giving birth and subsequent motherhood. It is also a reflection of the level of social anxiety.

According to research data, anxiety from moderate to high levels is one of the most important risk factors for development of anomalies in childbirth.

According to scientists, anxiety and fear lead to the production of stress hormones, and in pregnant women before childbirth the sensitivity to them is increased. Anxiety is accompanied by the release of catecholamines that interrupt the coordination of uterine contractions via binding β -adrenoreceptors that are located in the myometrium and, therefore, slowing down the progression of labour [6,7].

Almost all the literature available for referencing this issue [8,9,10] provides unidirectional data, according to which women with high levels of prenatal psychosocial stress are characterized by the complicated labour flow in the form of weakened childbirth. However, when analysing our data, we obtained completely opposite results (Table 2). For internally displaced women who gave birth in our maternity clinic, the opposite pattern was typical – each 5th case displayed rapid (precipitated) labour, and the incidence of weakness in labour in this group was 1.5 times lower than in the comparison group.

DISCUSSION

Reaction and response to any stress consists of 3 main components: emotional (disquiet, anxiety, fear), neuroendocrine (activation of the sympathoadrenal system) and behavioural. All of them are aimed at overcoming stress. But both: the action of extreme force stressors or chronic action of stressors cause the exhaustion (distress) of the body that subsequently somatises them. In women with high levels of psychosocial stress during pregnancy, we can clearly follow the said results during their labours.

Labours in women in this group can be characterized by 3 main features: rapid (precipitated) labour with high levels of obstetric traumatism (tear of perineum) and labour medicalization (EDA). The characteristics described above are due to such labour activity anomalies as hypertensive uterine dysfunction – the cases when the speed and strength of uterine contractions significantly exceed the normative parameters.

Also, this group of women was almost 1.5 times more likely to elect epidural anaesthetics due to painful contractions than in group 2, despite the fact they had regular labour activity on admission to the maternity clinic. The reasons for the high level of pain in labour in displaced women could be objective and subjective factors. Objective factors include excessive nociceptor irritation due to established cervical-uterine dysfunction. But the main reason is an impaired individual perception of pain. A high level of anxiety amplifies it at the level of the cerebral cortex. In addition, chronic stress leads to hyper-activation of the sympathetic division of the autonomic nervous system, which lowers the threshold of pain sensitivity.

For every woman, childbirth is an extreme stressful event that requires the mobilization of all body resources. Under normal stress reactivity and stress resistance, the body switches on physiological protective mechanisms that regu-

late and maintain normal labour; also natural mechanisms of anaesthesia are activated. Along with the active work of a woman in labour, the use of self-anaesthesia skills, her mobility, the availability of adequate support from a partner and medical staff, the favourable psychological factors are created to reduce individual sensitivity.

But instead of an active work displaced women avoid it by opting for passive pain management in the form of epidural anaesthesia.

In referencing the literature on the subject, we found single studies that study the hormonal regulation of emotions. In particular, Zelkowitz P., et al. in 2014 studied the relationship between the release of endogenous oxytocin during pregnancy and childbirth in women with psychosocial stress. The authors found high levels of this hormone in their blood. Endogenous oxytocin can be a buffer against the harmful effects of stress, protecting a woman from developing depressive symptoms and promoting more sensitive maternal behaviour [11]. That is, another reason for excessive labour can be the features of hormonal background in these women, but these data still need further study.

CONCLUSIONS

Labour activity in displaced women is characterized by hypertensive dysfunction with high levels of obstetric traumatism and medicalization during childbirth process. The prerequisites for its occurrence are chronic psychosocial stress, a reflection of which is a high and moderate level of anxiety. A mandatory standard for managing such pregnancy is psychological counselling and correction of the identified disorders. Given the high level of obstetric trauma in childbirth, it is necessary to foresee and provide the preventive measures in the form of remediation of inflammatory processes during pregnancy, gentle management of childbirth with the involvement of partner support and psychological support of the medical staff, the use of obstetric gels for labour and use of protective elements for the perineum.

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ORIGINAL ARTICLE

PAIN RELIEF DURING LABOR – WHAT DO WE KNOW AND WHAT SHOULD WE KNOW? RESEARCH BASED ON THE STUDENT POPULATION IN WARSAW

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The aim: Women of reproductive age often think of motherhood and labor with fear of intense labor pain. The anxiety they experience can lead to their postponing pregnancy. There are not many studies in the literature that research the knowledge young women have about the analgesia of labor. The aim of the present work was to evaluate the state of awareness about the possible methods of labor analgesia among women of childbearing age.

Material and methods: An Internet survey was conducted among 160 women. It consisted of questions on the pharmacological and non-pharmacological methods of labor analgesia. Most of the respondents were not medical university students (96.2%). Correct answers ranged between 11.3% and 97.5% of the total responses.

Results: The greatest number of incorrect answers were given to the question regarding contraindications to labor anesthesia (only 11.3% of answers were correct). There was also a low percentage of correct answers to questions about the risk of spinal cord injury during the procedure of inducing anesthesia (18.8% of correct answers), the motor activity of a woman after analgesia (22.5%) and the reimbursement of anesthesia (29.4%). Nearly 40% of the respondents did not know the correct answer to the question about the possibility of breastfeeding after anesthesia. The problem of analgesia during twin delivery also posed a challenge. Over half of the respondents (54.1%) incorrectly answered the question about the occurrence of complications among women who want to become pregnant again after the procedure of labor anesthesia. Moreover, 70.6% of the women surveyed considered non-pharmacological methods of labor anesthesia to be safer compared to pharmacological analgesia. The most commonly mentioned methods of non-pharmacological labor pain relief included breathing techniques and water birth.

Conclusions: The study shows that women of childbearing potential have an unsatisfactory level of knowledge about labor anesthesia. There is a need to improve the level of health education on this topic.

KEY WORDS: anesthesia, knowledge, pregnancy, perinatal care, health education

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INTRODUCTION

Every woman experiences labor in a unique way. The emotions that are often reported include excitement, fear, anxiety and uncertainty [1]. When women are asked about what they feel during childbirth, one of the most frequently given answers is pain [2]. According to the literature, labor pain can be one of the most painful experiences in the life of a woman [3]. It occurs in almost every woman in labor. Its intensity depends on many factors, such as the weight and birth length of the child, the size of the woman's pelvis, the position during labor and the individual pain threshold of a given woman [4]. There are also research results enumerating additional factors, such as the mental attitude of the mother, the course taken by labor, the level of the fear experienced and cultural features [5].

At this point it is important to provide the definition of pain, in which it is described as an "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" [6]. Labor pain is made up of two components: visceral pain and somatic pain. Visceral

pain occurs during the first stage of labor and is due to pressure exerted on the cervix. Somatic pain appears during both the first and the second stage of labor. It is caused by the pressure exerted on the cervix, the vagina and the perineum [7]. The pain experienced during labor impacts the respiratory system of the parturient leading to hyperventilation and an increase in oxygen consumption, which can result in respiratory alkalosis and a lowering of the blood volume transported to the fetus. In addition, pain can lead to a higher release of catecholamines and cortisol into the circulation [8].

Labor pain also has an important informational role about the stage of childbirth [9]. Uterine contractions become cyclical and their intensity gradually increases in duration, which is accompanied by a greater intensity of pain felt by the parturient [10]. It is believed that severe labor pain can have an adverse effect both on uterine function and the state of both the fetus and the mother [11].

In the scientific literature it is underlined that negative emotions felt during labor can lead to fear of the next time

childbirth would occur and can even lead to giving up becoming pregnant again. Fear of pain during subsequent labor can also increase the risk of postpartum depression [12]. Women of reproductive age report that thinking of motherhood is often accompanied by fear of the excessive intensity of labor pain. Many declare that they would like to avoid this pain and the fear they feel can lead to postponing the time of planning their pregnancy [13]. Not infrequently their experience of happiness and joy due to having a newborn baby is connected with negative emotions due to their memories of labor pain [14]. It is worth underlining that relieving labor pain is a mandatory component of pregnancy management [15]. Scientific studies underline the key importance played by the entire medical team and the childbearing women in alleviating intrapartum pain. Such cooperation guarantees better labor pain management and decreasing the risk of perinatal injuries, both in the mother and the child [14].

The availability of labor analgesia in Poland depends on the region of the country [16]. The methods of relieving labor pain can be divided into two groups. The first one includes three techniques: epidural analgesia, nitrous oxide administered by inhalation or intravenous opioids. Non-pharmacological methods providing pain relief during labor i.e. the second group include transcutaneous electrical nerve stimulation (TENS), breathing techniques, acupuncture, massage, aromatherapy or biofeedback [3].

The kind and safety of the analgesics provided are an important consideration for women of reproductive age and the education of parturients in this field later leads to an increased satisfaction with labor [10, 17]. The Polish scientific literature features few studies researching the knowledge of young women about pain relief during labor. International research revealed that the knowledge about analgesia, including epidural analgesia, is low. [18].

THE AIM

The aim of the present study was to define the level of knowledge on the pharmacological and non-pharmacological methods of relieving labor pain, particularly the awareness women of childbearing age have about the provision, contraindications, effects and possible complications of labor analgesia. The study also analyzed the factors which women consider when making the decision on the choice of labor analgesia.

The results that have been presented will make it possible for anesthesiologists and obstetricians to enhance their understanding of the fears experienced by pregnant women and will improve their contacts with their patients regarding issues related to the analgesia of labor.

MATERIAL AND METHODS

A group of students from the student research group called "Arkona" affiliated at the 1st Clinic of Anesthesiology and Intensive Care, Warsaw University of Medicine, created a survey made up of 33 closed questions. The questionnaire was prepared by medical students and anesthesiologists. The selection of questions

was supported by professional practice of anesthesiologists. Mini-Delphi was used as the method of structural selection. Its first part concerned socio-demographic data, while the second one consisted of questions regarding the pharmacological and non-pharmacological analgesic methods – with particular emphasis on labor analgesia. The survey was accessible for two months (1 December 2018 – 31 January 2019) to women studying at Warsaw universities on a well-known social media portal. During this time, 165 completed forms were collected. Analysis was conducted using the Fisher Exact Test. The value of $p < 0.05$ was considered to be statistically significant.

RESULTS

As a result of preliminary analysis, 5 incorrectly filled in questionnaires were rejected. The error was due to a lack of answers to one of the questions, which could have caused statistical calculation issues. Statistical analysis was conducted of the 160 correctly filled out forms. The respondents included solely women, mostly under the age of 25 (79.4%, $n=127$). The age between 25 and 35 years old was reported by 20% ($n=32$) of the respondents. Only one woman surveyed was over 35 years old. All of the respondents were university students. The majority came from cities with a population of over 150 thousand inhabitants. Such women composed 43.1% ($n=69$) of the total number of those surveyed, while 36.9% ($n=59$) came from towns with a population under 150 thousand, and 20% ($n=32$) indicated that their place of origin was a village (Table I).

The respondents were mostly women not studying at medical universities (96.3%, $n=154$), with 50% ($n=80$) of those surveyed studying at the Warsaw University of Life Sciences. The second most popular school was the University of Warsaw (28.1%, $n=45$).

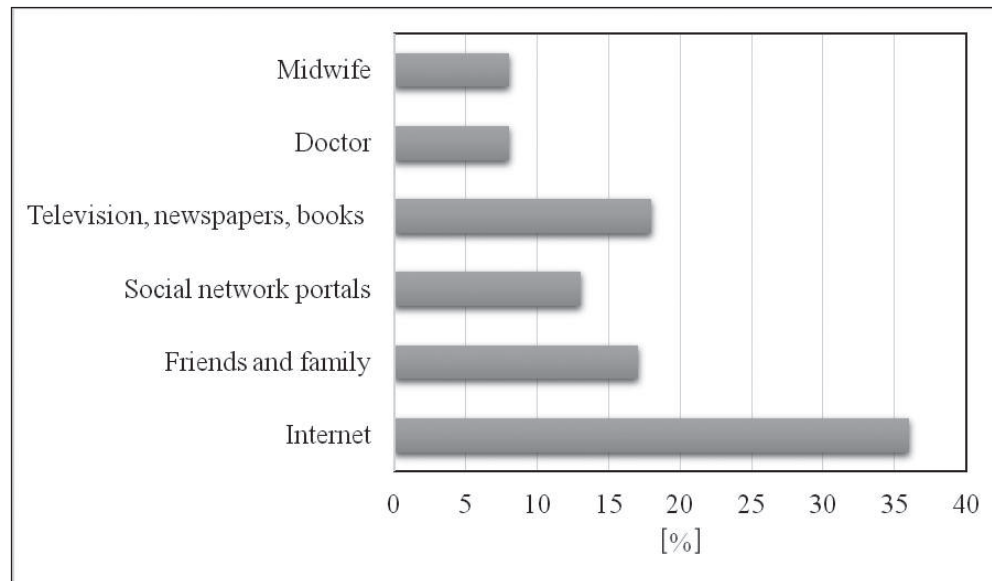
Before filling out the survey, the respondents evaluated their knowledge on labor analgesia on a scale of 0 to 10, with 0 indicating a lack of knowledge and 10 full knowledge of the topic. Both the median and the average of the answers was 4 ($SD=2.72$). Statistically more women under the age of 25 years old evaluated the level of their knowledge on labor analgesia as low (≤ 3) ($p=0.018$).

In response to the multiple-choice question concerning the source of their knowledge about labor analgesia, the most frequently indicated answer was the Internet (36%) as well as friends and family (17%). It is worth underlining that social media portals (13%), television, newspapers and books (18%) were indicated by a higher percentage of the respondents than a doctor (8%) or midwife (8%) (Fig. 1).

As many as 97.5% ($n=156$) of those surveyed claimed that labor pain can be relieved by using pharmacological or non-pharmacological methods. A similar number of women over 25 years of age gave the correct answer to this question. ($p=0.58$). Out of all those surveyed 47.5% ($n=76$) stated that pharmacological measures were safe for the baby. Women under the age of 25 more frequently gave a "yes" answer to this question ($p=0.003$). Regarding the safety of non-pharmacological techniques, 70.6% ($n=113$) thought that they were safe ($p=0.024$). This question re-

Table I. Demographic data.

FEATURE	NUMBER OF WOMEN SURVEYED	[%]
age	<25	127
	25-35	32
	>35	1
Place of origin	City over 150 thousand inhabitants	69
	Town 50-150 thousand inhabitants	17
	Town under 50 thousand inhabitants	42
	Village	32

**Fig 1.** Women's source of knowledge about labor analgesia.

ceived a positive answer from a similar number of women under 25 years old as those over that age ($p=0.833$).

The women surveyed were asked to make a hypothetical choice between a pharmacological and non-pharmacological method of intrapartum analgesia. Most of the respondents (61.9%, $n=99$) „decided” in favor of pharmacological analgesia. A similar number of those surveyed aged under and over the age of 25 chose this way of labor aesthesia ($p=0.844$). As many as 94.9% of those explained the reason for their decision by saying they expected the strongest pain relief effect from this kind of anesthesia. In the group of women who chose the non-pharmacological way of labor pain management the most frequently given reason for this choice was the low invasiveness of this kind of procedure (70.5%, $n=43$) and the safety of the child (21.3%, $n=13$). These reasons were statistically more frequent than in the case of pharmacological anesthesia ($p<0.001$).

The next part of the survey concerned knowledge about epidural labor anesthesia. The number of those surveyed that gave a “yes” answer to the question of whether the parturient remains conscious after being provided this kind of anesthesia was 76.9% ($n=123$). The respondents were also asked if epidural anesthesia limits the woman's motor activity. Out of all those surveyed 36.9% ($n=59$) answered “yes”. A lack of knowledge on this topic was declared by as many as 40.6% ($n=65$) of those surveyed ($p<0.001$).

The women taking part in the research were also asked about contraindications to epidural analgesia. 11.3% ($n=96$) of respondents incorrectly answered the question that possession of tattoos at the site of puncture for epidural anaesthesia is an absolute contraindication for this form of labour analgesia. 60% ($n=96$) of the respondents declared that they had no knowledge on this topic, and 28.7% ($n=46$) stated that tattoos were not a contraindication to this kind of anesthesia. The respondents were also asked if multiple pregnancy is a contraindication to epidural anesthesia. Only 3.4% ($n=5$) of the women incorrectly thought this was a contraindication, while 54.4% ($n=87$) declared a lack of knowledge on this topic. The difference between the correct answers to the two above questions was statistically significant ($p<0.001$). The next question regarded the age of the parturient. The age over 50 years is not contraindicated for epidural anaesthesia, which was correctly stated by 19.4% ($n=31$) of respondents. A lack of knowledge was declared by 59.4% ($n=95$) of the respondents and the “yes” answer was given by 21.3% ($n=34$) of the women ($p<0,001$). The survey also included a question on vitro fertilization as a potential contraindication to epidural anesthesia. The number of women who correct stated it was not a contraindication to epidural anesthesia was 58.8% ($n=94$). The rest did not answer this question.

The women surveyed were also asked about the potential complications of epidural labor analgesia. The number of

Table II. Knowledge of non-pharmacological methods of labor analgesia

Methods of relieving labor pain	[%]
Water birth	25
Breathing techniques	23
Manual methods (massage, hot and cold compresses)	18
Yoga	12
Music therapy	9
Aromatherapy	8
Acupuncture, acupressure	4
I do not know any non-pharmacological method of providing pain relief during labor	0.9
A TENS unit and transcutaneous electrical nerve stimulation	0.1

right answers that correctly induced anesthesia can lead to undesirable side effects amounted to 16.9% (n=27). Out of these respondents, when answering the more specific question regarding the possible complications of epidural intrapartum anesthesia, the greatest number of women (40.74%, n=11) incorrectly indicated stopping the progress of labor. Neurological complications, such as paresis and paralysis, were indicated by 29.63% (n=8), whereas headaches by 25.93% (n=7), so the two latter types of problems were thought to occur with similar frequency. The group of respondents foreseeing possible complications of epidural labor analgesia were also asked about the possible complications such as anesthesia can lead to neurological disorders in the newborn and potentially – a problem with getting pregnant again. These questions were correctly answered “no” by 81.48% (n=22) and 70.37% (n=19) of the respondents, respectively (p=0.416).

Moreover, the respondents had to answer a question regarding other methods of pharmacological anesthesia induced when alleviating intrapartum pain. Out of those surveyed, 48.1% (n=77) knew that one of such methods can be nitrous oxide in the form of inhalation. Moreover, 13.8% (n=22) of those surveyed correctly answered “yes”, when asked if opioid analgesics can be used (p<0.001).

The respondents were also asked a multiple-choice question on other methods of non-pharmacological anesthesia used for alleviating labor pain (Table II). The most frequently enumerated non-pharmacological methods of labor analgesia turned out to be water birth and breathing techniques. These were indicated by 25% and 23% of the respondents, respectively. Only 0.9% of those surveyed did not know any non-pharmacological method of labor pain analgesia.

DISCUSSION

According to the ordinance of the Minister of Health dated 9 November 2015 on medical procedure standards in pain management during labor, spontaneous labor analgesia is one of the integral parts of providing this health service and should include all the methods proven scientifically effective [19]. Epidural analgesia is considered the gold standard of providing pain relief during labor [20] respectively. The former revealed that only 47% of patients had been exposed to the concept of epidural analgesia in labour, and only 13%

opted for such analgesia. In the postnatal cohort, the overall epidural analgesia rate was 10%, although 19% had actually requested it. Patients who received epidural analgesia in labour were more likely to consider their experience as favourable (85%). In spite of the existence of clearly defined guidelines in this field, our study shows that many women are reluctant to use pharmacological methods of labor analgesia. They often hold scientifically unconfirmed views about dangers connected with using this kind of analgesia. At the same time, it is worth underlining that pregnant women often have no knowledge about the most frequent possible complications of this kind of analgesia. They give consent to anesthesia and obtain the information on the possible complications at a time when the discomfort due to labor makes it difficult for them to remember and analyze new information [21]. The questions we asked our respondents confirm the most common misconceptions about labour anaesthesia. The possession of tattoos at the site of puncture for epidural anaesthesia is not an absolute contraindication to this form of labour analgesia, as is the age of a woman over 50 years old, in vitro fertilization or multiple pregnancy. Contraindications include lack of consent of the patient, lack of cooperation with the patient, blood clotting disorders, shock, skin infection at the puncture site, some diseases of the central nervous system and some heart diseases and defects. Possible complications of epidural delivery anaesthesia include pressure drops, post-punk headaches, temporary pain in the lumbar region of the spine and extremely rare severe neurological complications such as meningitis or epidural hematoma [22].

Research conducted in European countries shows that women's views on pharmacological labor analgesia to a large extent depends on the organization of obstetric care and the opinions held by a given community on the role of pain in natural delivery. If there is a widespread social acceptance of natural delivery analgesia, pregnant women have a better attitude to pharmacological labor anesthesia [23]. In this context, it is worth quoting the results of a Polish survey on women's attitude to natural delivery. Almost half of the respondents indicated that their preferred method of pain relief during labor is epidural analgesia [24]. These results are in line with the data obtained in our study. When asked the question on the hypothetical choice between pharmacological and non-pharmacological pain management methods, over half

of those surveyed made the decision to use pharmacological methods of analgesia. Perhaps this is due to the belief that this kind of anesthesia is highly effective. Such responses also correspond with the observations of Irish researchers indicating that in the view of pregnant women the availability of pain relief is the most important factor influencing their positive attitude to natural delivery [25].

A survey on a large population of pregnant women in Hong Kong showed a low level of knowledge on the possible methods of relieving labor pain. Knowledge about the possibility to induce epidural anesthesia was definitely less widespread than in the population surveyed in our study, although epidural anesthesia was connected with a more positive experience of labor. It is worth underlining that the availability of this kind of analgesia for the respondents of the Hong Kong study was low, unlike in most European countries, including Poland. It is worth adding that the level of women's knowledge about epidural anesthesia has not changed in a significant way after they had actually experienced natural delivery [18]. This result corresponds with the data quoted above underlining how difficult it is to obtain conscious consent to epidural anesthesia once labor begins.

Research done in developing countries demonstrated a low level of awareness concerning methods of labor analgesia, even if those surveyed were well educated [26, 27]. The women surveyed in these countries often explain that the reason they do not want to have analgesia is because they want to fully experience natural delivery [28]. Such results are in contrast with the data obtained in our study, whose respondents first of all underlined the fear of dangerous complications connected with pharmacologically induced labor analgesia. It is worth adding that women in developing countries indicated that their main source of knowledge about pharmacological methods of labor analgesia was their doctor [29]. On the other hand, the results of our study were quite different. They clearly indicated that the women surveyed more often obtained information from the media, social media, their family and friends than from their doctor or midwife. This can be connected not only with greater access to different sources of information but also the fact that the participants of our survey were exclusively women with an incomplete university education.

To conclude, it is important to note that the pain connected with childbirth can contribute to the number of obstetric failure cases due to mechanisms connected with the increased level of cortisol and hyperventilation as well as respiratory alkalosis [30]. It is worth underlining that the lack of knowledge about the possible methods of labor analgesia and their effectiveness can additionally increase the level of fear and anxiety experienced by the parturient and can disrupt the course of natural delivery [25]. What is more, data from the literature clearly indicate that increasing women's level of awareness about the pharmacological and non-pharmacological methods of alleviating the level of labor pain (in connection with other methods) can lower the number of cesarean sections on demand by as much as 50%. In the group of women who have planned cesarean section on demand, every fourth one

would change her mind if she had access to epidural analgesia, while every tenth would make the decision in favor of natural delivery if she could benefit from non-pharmacological methods of relieving labor pain (mostly acupuncture) [8, 9, 10]. The data quoted in our study prove how important it is for the entire obstetric health care system that the level of pregnant women's awareness of labor pain analgesia in natural delivery should be raised.

Limitations: The study presented included only students studying in Warsaw took part in the presented survey. However, the majority of the respondents were students from only two Warsaw universities. This could have an impact on the extrapolation of the result for the entire population of reproductive-age women in Poland, but also in Warsaw.

CONCLUSIONS

The research shows that women of reproductive age, studying at Warsaw universities:

1. They have insufficient knowledge about anesthesia for childbirth. The conviction about high efficacy of pharmacological methods of labour anaesthesia, quite common among respondents, is not supplemented by education. In terms of safety, possible side effects and absolute contraindications to the use of these analgesia methods.
2. They consider non-pharmacological methods for the relief of labour pain to be safer but less effective than pharmacological methods.
3. The main sources of knowledge on labour anaesthesia are the media, family and friends. Much smaller physician and midwife.
4. When deciding about the methods of labour pain relief, women are guided by the expected strongest possible analgesic effect, low invasiveness of the procedure and safety of the child.
5. The level of knowledge about analgesia for childbirth is higher in the group of women over 25 years of age. This proves the need to increase the level of health education in labour anaesthesia, particularly in the group of women below 25 years of age, for whom the information on this subject may be particularly important in planning the date of first pregnancy. It should be stressed, however, that only female students of the Warsaw University were subjected to the study.

To sum up, the study demonstrated that in the education of women of childbearing age about labour anaesthesia, the focus should be on exposing the medical personnel as the source of knowledge, emphasizing the knowledge of safety, possible adverse effects and absolute contraindications for labour epidural anaesthesia, especially in women below 25 years of age.

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ORIGINAL ARTICLE

PROGNOSTIC INDICES OF PATHOLOGICAL TOOTH WEAR DEVELOPMENT

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ABSTRACT

The aim: To study possibilities of prognosis of pathological wear of tooth hard tissues development depending on the functional activity of masticatory muscles.

Materials and methods: In order to achieve the goal of the study, a survey of patients at the age of 19 to 69 years was conducted for precision of number of persons who have increased tooth wear. During the examination of all patients, three groups were selected: control and two researched ones. Patients in the control group (30 patients between the age of 18 and 60 years old) had an intact dentition without any visible sign of increased tooth wear.

The researched groups included 25 patients aged from 18 to 60 years old. The second research group included patients who had increased tooth wear of I-III level. The children of the patients of the II group with or without any sign of increased tooth wear were referred to the third research group.

Results: The method of electromyography was performed for the study of peculiarities of the muscular activity of the maxillofacial area of the patients of the control and research groups. In our opinion, all manifestations of increased tooth wear are related to changes in the muscle system, the motor apparatus and the nervous activity of an organism.

Conclusions: The study of direct muscular apparatus and related bone system may predict the possibility of developing of an increased tooth wear of young patients in the future and prevent its development, as well as to justify the application of a prevention and treatment plan of the studied pathology.

KEY WORDS: increased tooth wear, electromyography, tooth hard tissues, masticatory muscles

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INTRODUCTION

Tooth wear of hard tissues of teeth is a completely natural process, which has peculiarities in patients of different age groups. In some cases it is a slow compensated process, without any disturbance of physiological functions, in others it is a progressive process accompanied by severe teeth hyperesthesia, malfunction of masticatory function, malocclusion and changes in temporomandibular joint.

The process of abrasion of enamel and dentin occurs throughout the human life, and occurs as a result of regular contact between teeth themselves and between the teeth and food during the act of mastication, which may intensify, diminish over person's life and is considered physiological. Due to the mechanical strength of the enamel and dentin, both temporary and permanent teeth wear relatively slowly and evenly [1].

In young people, tooth hard tissue abrasion is less common than in middle-aged and elderly people. Frequency and reasons of wear of hard tissues of teeth in young people depend on both endogenous and exogenous factors, which negatively affect the stability of hard tissues of teeth and determine their abrasion [2, 3]. In elderly patients the reason of tooth wear development is a partial adentia (as a consequence of pathology of occlusion), malocclusion due to incorrect prosthodontic treatment and functional wear of dentures [4]. In women, tooth wear is slightly less common than in men.

In studies where patients were observed for a specific period of time with constant or repeated monitoring of

risk factors or health status, the researchers concluded that increased tooth wear at infancy predicts dental wear in adulthood. Insufficient development of enamel and dentin is considered to be the primary cause of tissue abrasion according to J. J. Pindborg (1970) [5].

THE AIM

To study possibilities of prognosis of pathological wear of tooth hard tissues development depending on the functional activity of masticatory muscles.

MATERIALS AND METHODS

In order to achieve the purpose of the study, a clinical examination of patients between the ages of 19 and 69 years was performed in order to identify the number of persons who have increased tooth wear. In all groups, both male and female patients were examined, who were equally matched in the groups. It should be noted that a greater number of male patients were between the ages of 40-49, that made 20% of the total number of patients in all groups. Majority of female patients were in the age group of 20-29 years, which also made 20% of total number of patients. This distribution of patients by age was reliable and did not affect the results of our study. During the examination of all patients, three groups were selected: control and two researched. Patients in the control group

Table 1. Electromyography results of patients from the control group (n = 30)

Masseter muscles	Researched parameters (M±m)			
	activity (msec.)	rest (msec.)	"K"	amplitude (mkV)
left	148,06±4,95	152,37±4,71	0,98±0,03	207,58±3,38
right	135,98±3,59	155,02±6,06	0,91±0,06	288,41±22,67

Note: 1. * – indicators of significance level between the left and right muscles $p < 0.05$;

2. n is the number of patients in the group.

(30 patients between 18 and 60 years old) had an intact dentition without any visible sign of increased tooth wear.

The ground of the research was based on the results of a survey of patients of two study groups, which were divided according to family relationships and disease manifestations in order to detect genetic susceptibility to increased abrasion in probands and features of pre-manifestations and manifestations of increased tooth wear.

The research groups included 25 patients aged 18 to 60 years old. The 2nd research group included patients with increased tooth wear of I-III stage. To the 3rd study group the children of patients of the 2nd study group with or without signs of increased tooth wear were attributed.

To study the peculiarities of the muscular activity of the maxillofacial area in patients of the control and researched groups, the method of electromyography was applied [6,7,8].

Electromyographic studies are based on the study of the potentials of action of muscle fibers, which function in the composition of mobile units, since they are a functional unit of activity of the neuromuscular apparatus [9,10].

For the myogram record a two-channel myograph M-440 company «Medicor», computer and printer were used. The record was carried out in the following mode: -calibration signal; – calmness; – jaw compression; – arbitrary mastication; – swallowing.

Such a program was used for examination of patients of all groups.

All electromyograms were analyzed by qualitative and quantitative indicators [8,9].

Evaluating qualitative indicators, we take in the account the presence or absence of activity during the rest, the nature of the excitation force at maximal closure of the jaws, the breakdown of the record structure, the unilateral or sequential nature of chewing, the uniform alternation of periods of bioelectric activity and rest of the muscles.

Quantitative data processing with the elements of variational statistics according to Student-Fisher was carried out using a computer program, which provided the definition of the following parameters:

- oscillation amplitudes (minimum and maximum, in μV), which characterize the excitation force and the number of motor units involved in muscle contraction;
- time of activity, as an indicator of concentration in the time of the process of excitation, rest – an indicator of the concentration of brake processes and one dynamic cycle (msec.);
- coefficient «K» – an indicator of the ratio of the processes of excitation and inhibition in each dynamic cycle – «activity» – «rest» [7,9].

For comparison of the obtained data the indicators of the control group were used, which correspond to the norm according to studies [10,11,12].

The idea of the state of masticatory muscles in patients with increased tooth abrasion and the nature of chewing function recovery can be created only on the basis of data on the nature of movements of masticatory muscles, their tone and bioelectric activity [8,9,10].

According to the purpose of the study, we received electromyograms in the control group with intact dental rows and in the patients of the researched groups before treatment.

RESULTS

Patients from the control group were examined on the qualitative and quantitative characteristics of the bioelectric activity of masticatory muscles.

In an electromyographic study of patients in the control group a functional test of «arbitrary mastication» was applied.

The intact dentition is characterized by the fact that the amplitude of the biocurrents on the working side is higher than on the balancing.

We paid attention to the main indices that characterize mastication in the researched patients, namely: activity of bioelectric volleys, rest time, activity factor «K» and amplitude of electromyogram. All these parameters were statistically processed using the Microsoft Excel.

Quantitative and statistical processing of electromyograms of control group persons are presented in the tab. 1

Analyzing the electromyogram digital data of control group we noticed that the activity and rest times of both the left and right masseter muscles are almost indistinguishable.

The activity of the left masseter was 148.06 ± 4.95 msec, while the right one was 135.98 ± 3.59 msec. The rest time of the left masseter was 152.37 ± 4.71 ms and the right muscle was 155.02 ± 6.06 ms.

It is noteworthy that the K activity factor for the left and right masseter muscles differs, respectively, at 0.98 ± 0.03 and 0.91 ± 0.06 , but this difference is not significant and indicates an absence of functional asymmetry.

There is a significant difference in left and right masseter muscle amplitude. The amplitude of the left one is $207.58 \pm 3.38 \mu V$, while the right one is only $288.41 \pm 22.67 \mu V$. This may indicate a more active movement of the right masseter muscle than the left one.

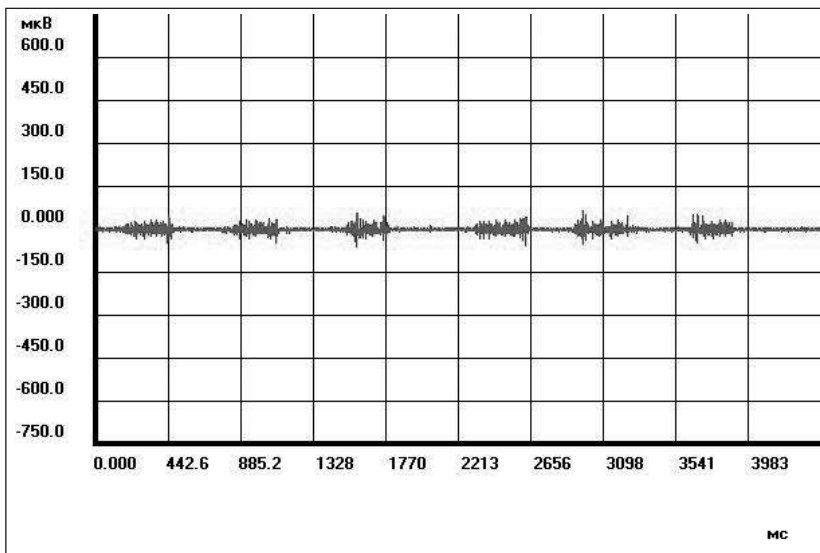


Fig. 1. Fragment of an electromyogram of the left masseter muscle of a patient 56years old before treatment (2nd group).

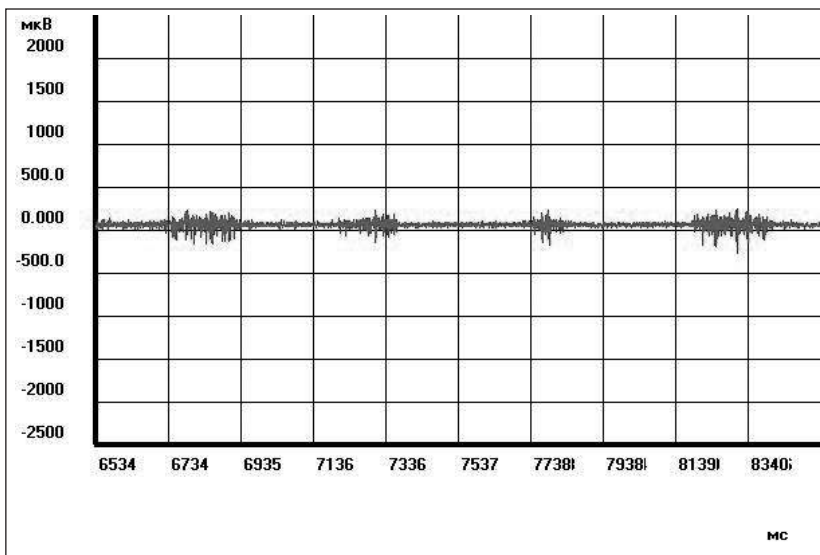


Fig 2. Fragment of an electromyogram of the left masseter muscle of a patient, 32 years old, before treatment (group 3)

Table 2. Electromyography results of patients 2nd group (n = 25) before treatment

Group	Masseter muscle	Parameters (M±m)			
		activity(mser.)	rest (msr.)	"K"	amplitude (мкВ)
2 (n=25)	left	226,1 ±24,89	327,46 ±22,84	0,71 ±0,049	383,45 ±42,41
	right	266,79 ±58,69	267,21 ±35,16	0,83 ±0,060	452,96 ±32,59

Notes: 1. * – indicators of significance level between the left and right muscles, $p < 0.05$;
2. n is the number of patients in the group.

Therefore, it can be concluded that, when performing masticatory movements, the muscles are capable to activate themselves instantly and transit rapidly to rest stage equally. The data obtained show that the muscles on both sides are not equally involved in the act of mastication and differ in functional asymmetry.

Indicators of electromyographic studies 2nd and 3rd research groups are different from the control group. In our opinion, this fact indicates a decrease in mastication efficiency due to the pathology of the hard tissues of the

teeth and a decrease in the height of the occlusion (Fig. 1, Fig. 2).

The results of the analysis of electromyograms of the second and third research groups of patients with increased tooth wear of I-III stage are presented in table. 2 and table. 3. Children of patients from the 2nd group who have had with or without signs of increased tooth wear were included to the 3rd group.

Comparing to the control group, the electromyography indices in the third group also differ on the left and right

Table 3. Electromyography results of patients from the 3rd group (n = 25) before treatment

Groups	Masseter muscle	Parameters (M±m)			
		Activity (msec.)	rest (msec.)	"K"	amplitude (mkV)
3 (n=25)	left	215,47 ±3,88	296,16 ±14,75	0,79 ±0,081	294,64 ±2,48
	right	279,18 ±31,95	285,07 ±32,31	1,11 ±0,09	354,43 ±36,69

Notes: 1. * – indicators of a significance level between the left and right muscles, $p < 0.05$;

2. n is the number of patients in the group.

sides (Fig. 2), but this difference is much smaller than in the patients of the second group (Tab.3).

In the 3rd group the coefficient of masticatory activity of the left masseter muscle was slightly higher comparing to the 2nd group (0.79 ± 0.081 vs 0.71 ± 0.049). In 3rd group, the activity coefficient of the right masseter muscle is much higher than in the 2nd group (1.11 ± 0.09 vs 0.83 ± 0.06 respectively) and the activity indicators of rest stage are slightly lower in the left master muscle in comparison to the right one.

Thus, the activity of the left masseter muscle in the 3rd group is 215.47 ± 3.88 ms, while the right one is 279.18 ± 31.95 ms.

The indices of the rest stage of left masseter muscle in the 3rd group were 296.16 ± 14.75 ms, while the right ones were 285.07 ± 32.31 ms.

It should be mentioned that the values of the amplitude of masseter muscles in the 3rd group are slightly lower than the corresponding indices in the 2nd group. Thus, the values of the amplitude of the left and right masseters in the 3rd group are respectively 294.64 ± 2.48 and 354.43 ± 36.69 μ V. These rates are significantly lower than the corresponding data obtained from the patients of the 2nd group.

Therefore, we suppose that differences in the electromyography parameters of 2nd and 3rd groups are related to the different degree of manifestation of increased tooth wear and malfunction of mastication.

All the manifestations of increased tooth wear, in our opinion, are related to changes in the system of muscles, motor apparatus and nervous activity of the body. Regarding to this, the study of the direct muscular apparatus and the related bone system may suggest the possibility of developing pathological wear of teeth of young patients in the future and prevent its development, as well as justify the use of a plan for the prevention and treatment of researched pathology.

CONCLUSIONS

Based on the results of our study, we can conclude that the study of the functional condition of the muscular complex is a reliable prognostic sign in the study of such a disease as an increased wear of hard tissues of teeth and is a reliable prognostic criterion for predicting the development of pathological abrasion of teeth of young patients in the future.

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ORIGINAL ARTICLE

EVALUATION OF PATIENT'S QUALITY LIFE WITH JOINT AND MUSCLE DYSFUNCTION

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ABSTRACT

The aim: was the evaluation the patient's quality of life with muscular and joint dysfunction of TMJ with using a modified profile questionnaire at various stages of orthopedic treatment. The study was conducted at the Department of Orthopedic Dentistry on the base of University Dental Center in Kharkiv National Medical University.

Materials and methods: We conducted a clinical examination and subjective analysis of 125 patients aged 20 to 60 years (mean age 38.5 ± 3), and 80 of them with a reliable diagnosis of dysfunction of TMJ and a control group of 45 patients of the same age without pathology of TMJ.

Our profile questionnaire is intended for filling by the respondent (patient) at the three control stages of orthopedic treatment under the supervision of an orthopedic dentist (before the start of treatment, 1.5 months after the beginning of treatment and 3 months). The data obtained were subjected to a scaling process, converted into percentages to facilitate statistical analysis.

Results: The worst values before and after treatment were found in the 3rd and 4th age subgroups (30.5 ± 0.60 and 42.0 ± 0.7 points, respectively), with a mean of 10 and 13%, respectively, according to the proposed rating scale. In this case, the indicators have changed 1.3 times in the direction of increase. The highest level of QL was observed in patients of the 2nd (98.7 ± 0.8 points) and the 1st subgroups (102.3 ± 0.4 points), the average value for the three stages of treatment was 78% and 92% rating scales. Before of orthopedic treatment, the values were approximately the same.

Conclusions: The using of profile questionnaire of quality of life in patients with muscular and joint dysfunction of the TMJ is appropriate. The questionnaire can be used to evaluate the dynamics of orthopedic treatment of this complex pathology, and it can also be used as a prognostic criterion for the end of the disease.

KEY WORDS: temporomandibular joint, muscular and joint dysfunction, analysis, quality of life, orthopedic treatment, modified profile questionnaire

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INTRODUCTION

The temporomandibular joint (TMJ) is one of the most actively working joints in human body. The complexity of the anatomical structure and biomechanics causes a high frequency of its dysfunction [1].

Dysfunction of the temporomandibular joint holds a special place among dental diseases due to the high prevalence, difficulties in diagnosis and treatment, a difficult and unusual clinical picture, which requires a differentiated approach of doctors of different profiles [2, 3]. As Sellmann H. (2003) noted, in the USA, the cost of treating temporomandibular joint dysfunction is after the treating of malignant tumors and has the second place be the prices of complex treatment.

In the scientific medical literature of recent years, there are articles about the need to change the established views on the diagnosis and treatment of TMG disorders because the emergence and development of this pathology is influenced by a variety of factors [1, 4, 5]. It could be the psycho-emotional felling of patients, traumas of the maxillofacial area, defects of the dental rows, which cause the dysfunction of chewing, the presence of fillings and dentures in the oral cavity, pathological abrasion of hard tissues of teeth, bad habits. Pain is one of the symptoms of the disease, and it can be so significant that patients have impaired function of chewing, swallowing and speech [6, 7].

The high attention is being to the need to expand cooperation not only between dentists of different specialties, but also with the cooperation of specialists in different fields of medicine with the obligatory assessment of psychosocial factors.

Many authors (Furberg C.D., Wiklund J., Herlitz J.) note, that it is now advisable to evaluate the effectiveness and cost-effectiveness of different therapies not only on the criteria that are widely used, but also on specific indicators of quality of life [8, 9].

Quality of life in medicine concerns, first and foremost, the state of health, although it is based on subjective perception and is a characteristic of respondents' in physical, psychological, emotional and social states. In most cases, modern medical research uses different methods of determining quality of life.

In nowadays in the world there are developed near 400 quality of life questionnaires. They are widely used in medicine, especially in those areas where the quality of life is most affected by disease [3, 10]. The analysis of the results makes it possible to characterize the condition of patients with different forms of pathologies. The most common include: EuroQOL (EQ – 5D) developed by European researchers, as well as American questionnaire SF 36 and its modifications (SF-22, SF-20, SF-12).

We can say that the assessment of the quality of life of patients is necessary not only to achieve the planned result

Table 1. Quantitative indicator of patients with muscular and joint dysfunction of TMJ by gender and age

		Quantitative indicator					
		Men		Women		Total	
Nº subgr	Age	dysfunction of TMJ	Without dysfunction of TMJ	dysfunction of TMJ	Without dysfunction of TMJ	dysfunction of TMJ	Without dysfunction of TMJ
1.	20-30 years	6	5	11	5	17	10
2.	30-40 years	7	5	12	4	19	9
3.	40-50 years	12	5	10	7	22	12
4.	50 -60years	10	6	10	8	20	14
Total		35 (28 %)	21 (16,8 %)	43 (34,4 %)	24 (19,2 %)	80 (65,4%)	45 (34,6 %)

of rehabilitation, but also to evaluate the effectiveness of the applied schemes and technologies of orthopedic treatment, which determined the purpose of our investigation.

THE AIM

Study of the evaluation the patient's quality of life with muscular and joint dysfunction of TMJ with using a modified profile questionnaire at various stages of orthopedic treatment.

MATERIALS AND METHODS

The study was conducted at the Department of Orthopedic Dentistry on the base of University Dental Center in Kharkiv National Medical University.

Deontological aspects are resolved taking into account the legislation in Ukraine, the Law of Ukraine «About Medicines», 1996, Art. 7, 8, 12, principles of ICH GCP (2008), order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 «On Approval of the Rules for Clinical Trials and Expertise of Materials of Clinical Trials and Model Regulations on the Ethics Commission», as amended; World Health Association Declaration of Helsinki. The study was performed with minimal psychological loss for patients. Patients were fully informed about the purpose and methods of the study, the potential benefits and risks, and the possible discomfort with the diagnosis and treatment. All ethical requirements for maintaining the confidentiality of the information received during the study are fulfilled. The work was reviewed and approved by the Bioethics Commission of the KNMU of the Ministry of Health of Ukraine.

We conducted a clinical examination and subjective analysis of 125 patients aged 20 to 60 years (mean age 38.5 ± 3), and 80 of them with a reliable diagnosis of dysfunction of TMJ and a control group of 45 patients of the same age without pathology of TMJ.

The main criteria for the selection of orthopedic patients in the study of quality of life were: gender-based distribution: men / women; age of patients – from 20 to 60 years; distribution into 4 groups according to the level of the disease and the etiological causes of its occurrence [11, 12].

To determine the social characteristics of patients and the level of their quality of life and creation of profile questionnaire, we used specific research methods, namely: asking information, formation and scaling of the results of the questionnaires; statistical methods of data processing (use of informative indexes); analysis and interpretation of the results.

Clinical information were obtained by conducting an extensive dental examination using the «Dental Status Assessment Card» according to WHO, 1995 [11, 13]. The result of the clinical examination was important for further diagnosis and choice of orthopedic treatment.

Assessed the presence of pain in the face, head and neck, in case of detection – took into account their localization, the timing and causes of occurrence, duration. Identified complaints of joint noise, its nature and causes. Psycho-emotional and general condition were evaluated.

In a state of physiological rest there was an examination of the patient in the central, anterior and lateral occlusions, as well as at the maximum opening of the mouth. The height of the lower third of the face, the severity of nasolabial and chin folds were evaluated. The position of the mandible was determined relative to the frontal, horizontal and sagittal planes. At the same time recorded the degree of mouth opening, winding movements, amplitude of the front and side movements of the jaw. [7, 14, 15].

Palpation of the TMJ was performed in a state of physiological rest, with the closure of the jaws in central occlusion, and during the movement of the mandible. The studies were performed externally in front of the goat ear, as well as through the external auditory canal. The presence of tenderness, the degree of excursion of the heads of TMJ, the synchrony of their movement, the presence of joint noise, the time of its manifestations and the nature of obstacles of sliding movement were evaluated.

The standardization of the data collection procedure was ensured by the unified nature of the survey and registration of epidemiological material using questionnaires: a general WHOQoL-100 quality of life questionnaire (WHOQoL Group, 1993) and a specialized dental quality questionnaire OHIP-14 (G. Slade, 1993) and our profile questionnaire [4, 12].

Table 2. Profile quality of life questionnaire for patient with muscular and joint dysfunction of TMJ

Questions			Criteria of answers			
1	2	3	4	5	6	7
Social box	Point criteria (5- point scale)	1 point	2 points	3 points	4 points	5 points
	How do you evaluate the quality of your life?	bad	unsatisfactorily	satisfactorily	good	perfectly
	How satisfied are you with your health?	bad	unsatisfactorily	satisfactorily	good	perfectly
	How healthy is the environment around you?	bad	unsatisfactorily	satisfactorily	good	perfectly
	How often have you had negative condition, such as bad mood, despair, depression?	always	often	sometimes	seldom	never
Psychosomatic box	Point criteria (5- point scale)	1 point	2 points	3 points	4 points	5 points
	Restrictions on chewing function	full	almost full	partial	temporary	not exist
	Physycal pain	always	often	sometimes	seldom	never
	Psychological discomfort	always	often	sometimes	seldom	never
	Physical disability	full	almost full	partial	temporary	not exist
	Psychological disability	full	almost full	partial	temporary	not exist
Social box	Social disability	full	almost full	partial	temporary	not exist
	Point criteria (5- point scale)	1 point	2 points	3 points	4 points	5 points
	Feeling weak, tired	always	often	sometimes	seldom	never
	Head pain	always	often	sometimes	seldom	never
	Pain in the parotid area	always	often	sometimes	seldom	never
	Swelling of the soft tissues of the parotid region	always	often	sometimes	seldom	never
	Tinnitus	always	often	sometimes	seldom	never
	Clicking in the joint	always	often	sometimes	seldom	never
	Difficulty opening the mouth	always	often	sometimes	seldom	never
	Oral hygiene	bad	unsatisfactorily	satisfactorily	good	perfectly
	Pain feelings during eating	always	often	sometimes	seldom	never
	Abrasion of teeth	always	often	sometimes	seldom	never
	Breaking of occlusion relationships	always	often	sometimes	seldom	never
	Breakage / correction of denrures	always	often	sometimes	seldom	never

RESULTS AND DISCUSSION

The distribution of examined patients was due to specific differences in the clinical features of the TMJ depending on the sex and different age periods, the data of which are presented in Table 1.

The quantitative indicators are shown as follows: men with TMJ dysfunction – 35 patients (28% of the total number of examined), women with dysfunction of the TMJ – 43 patients (34.4% of the total number of surveyed), men without pathology of the TMJ – 43 patients (34.4%

Table 3. Conversion Scale: Quality of Life Assessment of Patients with TMJ muscular and joint dysfunction

№	Result (total point for one examination)						Total		Result
	Before treatment		After 1,5 months of starting treatment		After 3 months of starting treatment				
	Point	%	Point	%	Point	%	Point	%	
1	100-110	30%	100-110	30-35%	100-110	40%			A
2	88-99	20-25%	88-99	20-25%	88-99	30-35%			B
3	66-87	20-12%	66-87	35%	66-87	20-30%			C
4	44-65	15%	44-65	15-20%	44-65	25%			D
5	22-43	5-10%	22-43	5-10%	22-43	10%			F

of the total number of examined), women without the pathology of the TMJ – 19 patients (21.2% of the total number of patients).

It is important to note that patients of group 1 were selected with dysfunctions, which were caused in most cases by psycho-emotional disorders, spine diseases, patients of group 2 – diseases of spine, arthritis, arthrosis. Group 3 had a history of occlusion disorders and arthritis.

Examining these complaints and conducting the questioning of the investigated patients, we created a sample of questions that reflected the actual quality of life problems of orthopedic treatment of dysfunction of the TMJ, the contents of which are presented in our modified profile questionnaire in Table 2.

Our profile questionnaire is intended for filling by the respondent (patient) at the three control stages of orthopedic treatment under the supervision of an orthopedic dentist (before the start of treatment, 1.5 months after the beginning of treatment and 3 months after the beginning of treatment). The data obtained were subjected to a scaling process, converted into percentages to facilitate statistical analysis. The answers to the questions were evaluated as a percentage, according to the result conversion scale shown in Table 3.

The testing phase was accompanied by a preliminary psychometric evaluation of the questionnaire: assessment of the frequency distribution of answers to the questions; reliability assessment (Cronbach's coefficient on questionnaire scales); assessment of construct validity (analysis of questionnaire factor structure and correlation relationships within its structure). In compliance with all the above requirements, the final version of the questionnaire was formed, taking into account the results of the pilot testing.

The answer to each question, depending on the severity of the situation was evaluated in the number of points from 1 to 5 (from the lowest degree to the highest).

The results of the first test showed that, in general, all the questions and answer options were clear to the patients and did not cause much difficulty in the examination. The average length of time a respondent completed the profile questionnaire was up to 15 minutes.

The worst values before and after treatment were found in the 3rd and 4th age subgroups (30.5 ± 0.60 and 42.0 ± 0.7 points, respectively), with a mean of 10 and 13%, respec-

tively, according to the proposed rating scale. In this case, the indicators have changed 1.3 times in the direction of increase. The highest level of QL was observed in patients of the 2nd (98.7 ± 0.8 points) and the 1st subgroups (102.3 ± 0.4 points), the average value for the three stages of treatment was 78% and 92% rating scales. Before of orthopedic treatment, the values were approximately the same.

CONCLUSIONS

The analysis of our investigations shows that the use of a profile questionnaire of quality of life in patients with muscular and joint dysfunction of the TMJ is appropriate. The questionnaire can be used to evaluate the dynamics of orthopedic treatment of this complex pathology, and it can also be used as a prognostic criterion for the end of the disease.

In our patients of the general sample, the average score increased in the short term (1.5 months) after the start of orthopedic treatment and the values significantly changed towards improvement after 3 months, indicating a positive dynamics and correctness in the choice of orthopedic treatment for patients with muscular and joint dysfunction of the TMJ at the clinic of orthopedic dentistry.

In the treatment of patients of 1 age subgroup, the result of which was (93.38 ± 1.19 and 89.3 ± 2.6 points); also patients of the 4th subgroup (102.3 ± 0.4 points). Long-term outcome of prosthetics, namely after 3 months of orthopedic treatment (102.4 ± 0.5 and 106.8 ± 1.9 points) and (108.86 ± 0.9 points), which indicates a significant improvement in quality of life patients.

The dependence of quality of life on orthopedic treatment for with muscular and joint dysfunction of the TMJ is significant, and treatment, in turn, leads to an improvement in the quality of life of the patients. That is why the results of investigations presented in this article make it possible to practically use the questionnaire in assessing the quality of treatment in the practice of orthopedic dentists.

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ORIGINAL ARTICLE

ANALYSIS OF CYTOKERATINE 19 EXPRESSION LEVEL BY IMMUNOCYTOCHEMICAL DIAGNOSTIC METHOD IN LARYNGEAL CANCER PATIENTS WITH REGIONAL METASTASES

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ABSTRACT

The aim: To investigate the informative content of immunocytochemical studies of lymph node aspirate using a molecular marker of cytokeratin 19 in patients with laryngeal cancer as for regional metastatic spread.

Materials and methods: A comprehensive investigation with immunohistochemical study of squamous cell carcinoma of larynx of 74 patients with laryngeal cancer II – IV stage (T2–4 N0–3 M0) of the second clinical group was conducted.

Results: Statistically significant differences ($p < 0.001$) in the structure of distribution of marker expression between groups were revealed. Thus, a marked expression of cytokeratin 19 was determined in 11 (29.73%) representatives of the group with regional metastases and only in 1 (2.7%) patient without tumor metastases ($p = 0.002$). In the group with metastases the presence of atypical cells was confirmed cytologically in 26 cases (70.27%).

Conclusions: To predict the development of laryngeal metastases in laryngeal cancer patients, it is recommended to carry out an immunocytochemical study using a diagnostic marker of cytokeratin 19. The positive status of cytokeratin 19 increases the chances of regional metastasis of laryngeal cancer by 18.37 times ($p < 0.001$) compared with absence of expression.

KEY WORDS: laryngeal cancer, immunocytochemical study, cytokeratin 19

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INTRODUCTION

Laryngeal cancer is the leader in malignancies of the upper respiratory tract. Despite the progress in world medicine, the survival rate of laryngeal cancer patients has not changed much over the last 20 years. [1,2] Regional metastases into the cervical lymph nodes, the value of which exceeds the size and localization of the primary tumor, play a major role in the prognosis of the disease and are second only to the presence of distant metastases. [3]

The evolution of regional metastasis treatment has gone from a radical operation first described in 1888 to superselective interventions having been performed in recent years. [4,5] Discussion of the volume of cervical dissection is reflected in many publications, which proves the urgency of the problem. [6]

Ultrasound-guided fine needle aspiration biopsy is a standard for the study of the metastatic status of lymph nodes. [7] However, the organ-preserving trend in oncology requires the search for new methods of diagnosing metastatic lymph nodes to accurately select the volume of intervention. Much attention is paid to the study of the expression of molecular markers. In recent years, oncology has paid considerable attention to immunohistochemical, immunocytochemical studies and molecular tumor markers. [8]

The cytoskeleton of all cell types is determined by a set of intermediate filaments, microtubules, and microfilaments. Cytokeratins belong to the group of intermediate filaments, which also include vimentin, desmin, neurofilaments, laminin and glial fibrillar protein. They are characteristic filaments for both epithelial cells and the corresponding epithelial tumors and, due to their high specificity and sensitivity, are used to diagnose the latter. [9]

Nowadays, given the high mortality rate in the first year after diagnosis, treatment and diagnosis of laryngeal cancer, and in particular of its metastases, requires further research, development and implementation of new methods in practice [10,11].

THE AIM

To investigate the informative content of immunocytochemical studies of lymph node aspirate using a molecular marker of cytokeratin 19 in patients with laryngeal cancer as for regional metastatic spread.

MATERIALS AND METHODS

To perform the research tasks, 74 patients with laryngeal cancer stage III – IV (T3–4N0–3M0) and II clinical group underwent examination, treatment and follow-up in the ENT-Oncology Department of I.I. Mechnikov Dniprop-

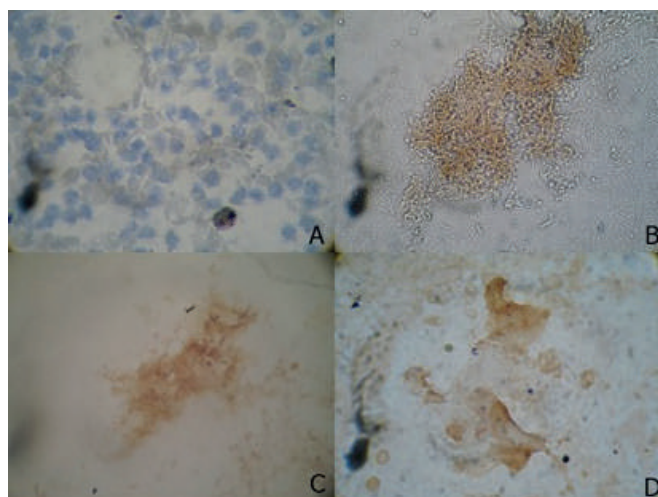


Fig. 1. Defining of cytokeratin 19 expression by immunocytochemical study; microslide stained with monoclonal antibodies cytokeratin 19 (x100). A. - no expression (0%); B. - weak expression level 11-20%; C. - moderate expression level (21-50%); D. - high expression level (>50%).

etrovsk Regional Clinical Hospital from 2011 to 2015. In 37 patients regional metastases of laryngeal cancer were diagnosed and histologically confirmed, the other 37 patients without metastases represented a comparison group.

Transcutaneous aspiration biopsy under ultrasound control was performed in all patients, the obtained cytological preparations were fixed with Cytospray.

The material of the aspirate was examined by cytological and immunocytochemical method. Monoclonal antibodies to cytokeratin 19 (clone RCK108 Dako) were used for the study. For each marker, control studies were performed to eliminate false positive or false negative results. The specimens were examined by light microscopy on a Carl Zeiss microscope. The assessment of marker expression was made with a semi-quantitative method with the following levels: absence of expression – 0-10%, weak – 11-20%, moderate – 21-50% and marked – > 50%.

Modern mathematical and statistical methods of descriptive and analytical statistics were used for the statistical processing of the obtained material, with results in the form of: number of observations (n); arithmetic (M), standard deviation (SD), 95% of confidence interval (95% CI), median (Me), and interquar-

tile range (25-75% percentile). Methods of statistical analysis included: checking the distribution of quantitative attributes for compliance with the normal law by the Shapiro-Wilk test; estimation of probability of differences under conditions of normal distribution by two-sided t-test (Student test), in the other case – by Mann-Whitney test (U test), categorical variables – by Pearson Chi square (χ^2) (Pearson Chi-square), including Yates Chi-square correction for continuity; estimation of the association between categorical variables using Cramer's V (Cramer's V) and Spearman's rank correlation coefficients (ρ); calculation of odds ratio (OR); ROC-analysis.

The data were processed using Statistica v.6.1 Statistical Analysis License Packs. There was studied the level of expression of the molecular marker of cytokeratin 19 by the immunocytochemical method in lymph nodes of 74 patients with stage III-IV cancer. Among the examined, all patients were male, 59.46 – 63.45 years old (95% CI), with no statistically significant differences between groups ($p > 0.05$) in age and degree of tumor differentiation (Table I).

RESULTS

In total, cytokeratin 19 expression was absent in 37 patients (50.0%), low expression was in 12 patients (16.22%), moderate – in 13 (17.57%), and marked – in 12 (16, 22%) (Fig. 1, Table II).

In the group of patients with regional metastases ($n = 37$), a positive status of cytokeratin 19 was observed in 30 (81.08%) patients. Of these, a weak reaction was in 8 (21.62% of the total number of patients in the main group), moderate – in 11 (29.73%) and strong – in 11 (29.73%). In the comparison group, expression of cytokeratin 19 was detected in 7 patients (18.92%): weak expression – in 4 patients (10.81% of the total number of patients in the comparison group), moderate – 2 (5.41%), marked expression was observed in 1 patient (2.7%); expression was absent in 30 (81.08%) patients. Statistically significant differences ($p < 0.001$) in the structure of distribution of marker expression between groups were revealed (Table 2). Thus, a marked expression of cytokeratin 19 was determined in 11 (29.73%) representatives of the group with regional metastases and only in 1 (2.7%) patient without tumor metastases ($p = 0.002$).

In the group with metastases the presence of atypical cells was confirmed cytologically in 26 cases (70,27%).

Table I. General characteristics of the examined patients with laryngeal cancer

Characteristics	All examined	Main group with regional metastases	Comparison group without regional metastases	p
Number of patients, n (%)	74 (100 %)	37 (50,0 %)	37 (50,0 %)	-
Age (years)				
M (SD)	61,46 (8,61)	59,84 (7,01)	63,08 (9,79)	0,106*
95 % CI	59,46 - 63,45	57,50 - 62,17	59,82 - 66,35	
Degree of tumor differentiation, n (%)				
High G1	14 (18,92 %)	6 (16,22 %)	8 (21,62 %)	0,553
Moderate G2	51 (68,92 %)	27 (72,97 %)	24 (64,86 %)	0,451
Low G3	9 (12,16 %)	4 (10,81 %)	5 (13,51 %)	0,722

Notes. p - Differences between groups according to Pearson's criterion χ^2 ;

* - by Student's t test

Table II. Expression of cytokeratin 19 in lymph nodes depending on the presence of metastases

Level of expression	All examined (n=70)	Main group (n=37)	Comparison group (n=37)	p
Moderate level, Me (25 %;75 %)	11,22 (5,79; 27,50)	27,50 (12,50; 52,50)	6,58 (3,19; 9,44)	<0.001*
Distribution of level of expression, n (%)				
No expression (0-10 %)	37 (50,0 %)	7 (18,92 %)	30 (81,08 %)	<0.001
Weak expression (11-20 %)	12 (16,22 %)	8 (21,62 %)	4 (10,81 %)	0.207
Moderate expression (21-50 %)	13 (17,57 %)	11 (29,73 %)	2 (5,41 %)	0.006
Marked expression (>50 %)	12 (16,22 %)	11 (29,73 %)	1 (2,7 %)	0.002
Differences and associations between groups**	$\chi^2=30,19$ (p< 0.001); V=0.64; $\rho=0.63$ (p< 0.001)			-
High G1 degree of tumor differentiation				
Moderate level, Me (25 %;75 %)	9,24 (7,47; 17,50)	37,50 (12,50; 57,50)	7,50 (5,92; 9,24)	0.028*
Distribution of level of expression, n (%)				
No expression (0-10 %)	8 (57,14 %)	1 (16,67 %)	7 (87,5 %)	0.035
Weak expression (11-20 %)	3 (21,43 %)	2 (33,33 %)	1 (12,5 %)	0.778
Moderate expression (21-50 %)	0 (0%)	0 (0%)	0 (0%)	-
Marked expression (>50 %)	3 (21,43 %)	3 (50,0 %)	0 (0%)	0.110
Differences and associations between groups**	$\chi^2=7,70$ (p= 0.021); V=0.74; $\rho=0.74$ (p= 0.002)			-
Moderate G2 degree of tumor differentiation				
Moderate, Me (25 %;75 %)	12,50 (5,18; 27,50)	27,50 (12,50; 52,50)	6,19 (2,99; 9,85)	<0.001*
Distribution of level of expression, n (%)				
No expression (0-10 %)	24 (47,06 %)	5 (18,52 %)	19 (79,17 %)	<0.001
Weak expression (11-20 %)	8 (15,69 %)	5 (18,52 %)	3 (12,5 %)	0.838
Moderate expression (21-50 %)	11 (21,57 %)	9 (33,33 %)	2 (8,33 %)	0.068
Marked expression (>50 %)				
Differences and associations between groups**	$\chi^2=21,02$ (p< 0.001); V=0.64; $\rho=0,64$ (p< 0.001)			-
Low G3 degree of tumor differentiation				
Moderate level, Me (25 %;75 %)	8.85 (4,65; 27,50)	22,50 (13,17; 32,50)	4,65 (0,65; 8,05)	0.178*
Distribution of level of expression, n (%)				
No expression (0-10 %)	5 (55,56 %)	1 (25,0 %)	4 (80,0 %)	0.330
Weak expression (11-20 %)	1 (11,11 %)	1 (25,0 %)	0 (0%)	0.906
Moderate expression (21-50 %)	2 (22,22 %)	2 (50,0 %)	0 (0%)	0.324
Marked expression (>50 %)	1 (11,11 %)	0 (0%)	1 (20,0 %)	0.906
Differences and associations between groups**	$\chi^2=5,76$ (p=0.124); V=0.80; $\rho=0,38$ (p=0.311)			-

Notes. p - differences between groups according to Pearson criterion χ^2 , including Yates' correction;

* - by criterion U of Mann-Whitney;

** - associations by Cramer V-criterion (Cramer's V) and Spearman correlation coefficient (ρ)

DISCUSSION

At different degrees of tumor differentiation in laryngeal cancer patients with metastatic neoplasm, the proportion of patients without cytokeratin 19 expression was sig-

nificantly higher than among patients of the comparison group, without statistically significant differences only at a low degree (p=0.035 in G3, p<0.001 in G2 and p=0.124 in G3).

Table III. Assessment of prognostic potential of cytokeratin 19 for regional metastasis of laryngeal cancer depending on the degree of tumor differentiation according to ROC-analysis

Characteristics	Se, %	Sp, %	AUC (95 % CI AUC)	p	Assessment AUC	Optimal cut-off point
Degree of tumor differentiation n (%)						
High G1	83,33	87,50	0,865 (0,581 - 0,985)	0,004	very good	>9,42 %
Moderate G2	81,48	79,17	0,840 (0,710 - 0,927)	<0,001	very good	>9,94 %
Low G3	100,00	80,00	0,800 (0,422 - 0,979)	0,131	very good	>8,05 %
Total	81,08	81,08	0,841 (0,737 - 0,916)	0,134	very good	>9,94 %

Notes. Se - Sensitivity;
Sp - Specificity;
AUC - Area Under ROC Curve;
p - level of significance of AUC;
AUC - quality assessment of predictability of marker;
Optimal cut-off point - the optimal cut-off point

Moderate level of marker expression also did not differ in the comparison groups with a low degree of tumor differentiation ($p=0.178$). In cases of moderate and high degree of differentiation, it was significantly higher in the group of patients with metastases compared with patients without metastases ($p < 0.001$ and $p=0.028$, respectively). Overall, a moderate level of expression of cytokeratin 19 in laryngeal cancer patients with metastases was 27.50% (12.50; 52.50) – Me (25%; 75%), whereas in the group without metastases – 6.58% (3.19; 9.44), $p < 0.001$; the difference in median marker values in the comparison groups is 18.14%, 95% CI (10.03 – 25.03).

An evaluation of the association between metastases and the level of cytokeratin 19 expression in lymph nodes according to Cramer criterion ($V=0.64$) and Spearman's rank correlation coefficient ($\rho = 0.63$ $p < 0.001$) showed a significant relationship between them as a whole in all examined and in patients with moderate and high degree of tumor differentiation. This is an evidence of the significant diagnostic value of the expression of this molecular marker in the detection of regional dissemination of tumor. To assess the ability of cytokeratin 19 to predict regional dissemination of tumor in patients with laryngeal cancer, ROC analysis was performed (Fig. 2, Table III).

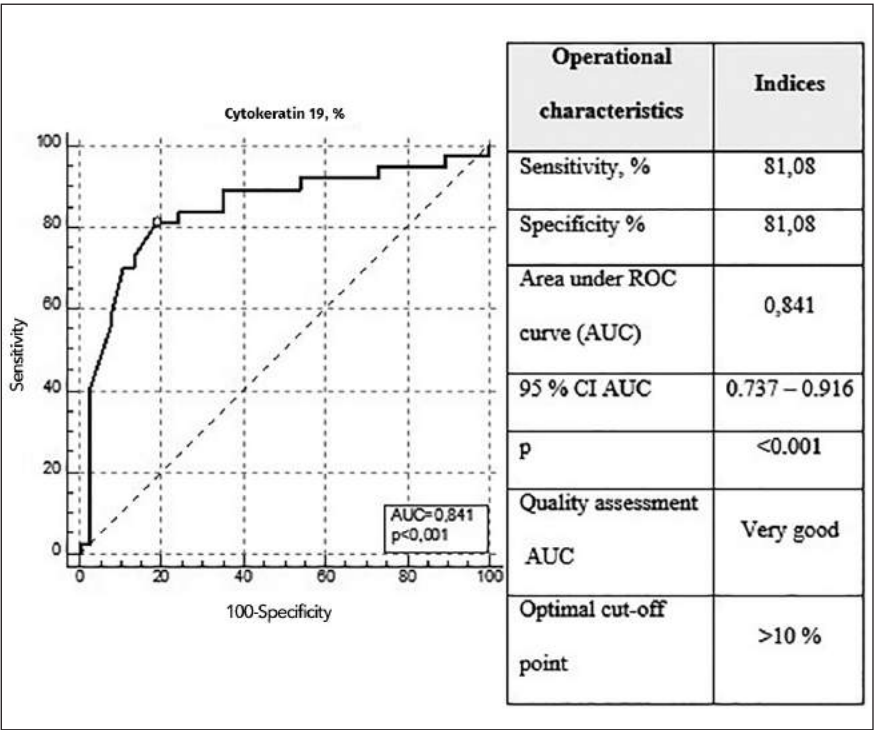


Fig. 2. Assessment of the possibility of predicting regional metastases of laryngeal cancer based on the level of cytokeratin 19 expression in lymph nodes according to ROC-analysis.

Statistically significant ($p < 0.001$), very good operational characteristics in cytokeratin 19 for the prediction of regional metastases of laryngeal cancer were found: sensitivity – 81.08%; specificity – 81.08%; area under the ROC curve AUC=0.841, 95% CI (0.737 – 0.916).

An assessment of the prognostic potential of cytokeratin 19 for regional laryngeal cancer metastases showed that this marker had very good operational characteristics regardless of the degree of tumor differentiation (no statistically significant AUC was obtained for low tumor differentiation).

These results of ROC-analysis suggest that prediction of regional laryngeal cancer metastases is possible on the basis of cytokeratin 19 expression. The critical value of the marker expression level by the optimal cut-off point is $> 9.94\%$, i.e. a weak expression level.

In weak and higher levels of cytokeratin 19 expression patients have a higher chance of regional metastasis of laryngeal cancer – the odds ratio OR is=18.37 (95% CI 5.74 – 58.79; $p < 0.001$).

CONCLUSIONS

1. In immunocytochemical study of the expression level of cytokeratin 19 in patients with squamous cell laryngeal cancer, positive expression was determined in 37 (50.0%) patients with a low level – 11.22% (5.79; 27.50); in more than a third of patients with moderate (13 patients – 17.57%) and marked (12 patients – 16.22%) expression of the marker.
2. In laryngeal cancer patients with regional metastases, the values of expression of the molecular marker were 18.14% higher (95% CI 10.03 – 25.03) by median values.
3. The diagnostic value of cytokeratin 19 expression in relation to regional metastasis was determined by Cramer criterion ($V = 0.64$), Spearman correlation coefficient ($\rho = 0.63$, $p < 0.001$), ROC- analysis (very good operational characteristics: sensitivity – 81.08%; specificity – 81.08%; AUC = 0.841, $p < 0.001$).
4. To predict the development of laryngeal metastases in laryngeal cancer patients, it is recommended to carry out an immunocytochemical study using a diagnostic marker of cytokeratin 19. The positive status of cytokeratin 19 increases the chances of regional metastasis of laryngeal cancer by 18.37 times ($p < 0.001$) compared with absence of expression.
5. The prospects for further research are aimed at investigating additional molecular markers for determining optimal therapeutic and diagnostic tactics in regional laryngeal cancer metastases.

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ORIGINAL ARTICLE

A TEN-YEAR ANALYSIS OF CHANGES IN THE SENSITIVITY OF THE LEADING UROPATHOGEN TO ANTIBACTERIAL AGENTS IN CHILDREN WITH URINARY TRACT INFECTION IN THE NEPHROLOGY DEPARTMENT

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ABSTRACT**The aim:** To study the prevalence of ABR among children with UTI over the past 10 years, with an assessment of the sensitivity of *E. Coli* to common antibiotics in dynamics.**Materials and methods:** The study involved 1,044 children with UTI aged from 1 month to 18 years. Examination of patients and interpretation of the results was performed in accordance with the provisions of the Declaration of Helsinki of Human Rights. According to the design, the study included 3 comparison groups: Group 1 — children of the 2009 follow-up year ($n = 337$), Group 2 — of 2014 ($n = 328$) and Group 3 — of 2019 ($n = 379$).**Results:** *Escherichia coli* is recognized as the leading uropathogen in all study groups: its percentage in Group 1 was 47 % (158/337), in Group 2 — 64 % (210/328) and in Group 3 — 66.5 % (252/379). The prevalence of antibacterial resistance of *E. coli* strains and the high dynamics of its growth are shown. So the level of resistance of *E. Coli* in 2019 was 70 ± 4.06 % (176/252). This was 11 % more compared to 2014 and 18.8 % more compared to 2009. The percentage of multiresistant strains tended to increase and amounted to 28 ± 9.97 % (70/252) among patients with UTI and 40 ± 9.12 % (70/176) in the structure of resistance in 2019. The relative risk of ABR increased by 1.6 times in 2019 compared to 2014 ($RR_{2019} = 2.208 \pm 0.207$ [1.473;3.310], $p < 0.05$ vs $RR_{2014} = 1.375 \pm 0.209$ [0.913;2.063]) and by 3 times compared to 2009 ($RR_{2009} = 0.727 \pm 0.209$ [0.483;1.095]). Ampicillin and amoxicillin showed an equally low sensitivity level (3.5 ± 32.14 % (9/252)). Only every second child confirmed sensitivity to cefuroxime (53.6 ± 5.76 (135/252)). Ceftazidime and ciprofloxacin showed a relatively high sensitivity level — 77.4 ± 3.34 (195/252) and 83 ± 2.81 (209/252), and at the same time the rapidly growing resistance rates — almost twice as high over the past 5 years. Furazidin K showed a high sensitivity level of 85.7 ± 2.53 % (216/252), the lowest level of overall resistance of 14.3 ± 15.15 % (36/252) and a slow rate of its formation. An unfavourable prognosis of an increase in the relative risk of ABR by 2.9–3.7 times in the next 5–10 years was determined among patients with UTI, provided that the existing diagnostic and treatment approaches are maintained.**Conclusions:** The study results are important for understanding the clinical decision on the benefits of antibacterial therapy and optimizing its empirical choice for a patient with UTI.**KEY WORDS:** urinary tract infection, children, *E. Coli*, antibiotic resistance

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INTRODUCTION

Selection of drug-resistant strains of pathogens is an integral part of modern antibacterial therapy [1]. Antimicrobial resistance is becoming a big risk to the global health of mankind [2].

Urinary tract infection (UTI), as one of the most common bacterial infections in children with an empirical antibiotic treatment strategy, is of particular multifaceted relevance [3]. *Escherichia coli* in childhood plays an important role as an infectious factor, as it is the cause of more than 80 % of all UTIs, bacteremia, foodborne infections, meningitis in newborns and other life-threatening conditions [4].

Recent studies have shown that up to half of *E. Coli* culture samples in children with urinary tract infections (UTI) are resistant to common antibiotics [5]. Researchers from the University of Bristol and Imperial College London studied antibiotic resistance in UTI caused by *Escherichia coli* and showed a growing level of its

resistance to β -lactam antibacterial agents (ampicillin, amoxicillin, including protected by clavulanic acid) [6]. In other developed countries, a third of *E. Coli* samples were resistant to co-trimoxazole, and a quarter — to trimethoprim. In developing countries, resistance was even higher, probably due to the availability of over-the-counter antibiotics, according to the authors [7, 8]. Researchers point to the broad regular prescription of antibiotics by primary care physicians, which poses a high risk of resistance in children, which can persist up to 6–12 months after treatment [5, 9].

So, on the one hand, children receive a disproportionately large number of antibiotics compared to other segments of the population, on the other hand, they are more vulnerable to immediate and long-term complications, such as renal scarring and renal impairment in inadequate treatment of UTI [10]. Constant monitoring of local microbial sensitivity is an absolute necessity for the correct empirical choice of antibiotic.

Table I. Dynamics of *E. coli* sensitivity in patients with UTI for the period 2009 - 2019.

Antibiotics	Strains <i>E.coli</i>	Years		
		2009 % ± 95% CI, (abs.)	2014 % ± 95% CI, (abs.)	2019 % ± 95% CI, (abs.)
Ampicillin	S	20±15,06 (34/168)	30,5±10,24* (64/210)	3,5±32,14* (9/252)
	MR	28,6±11,99 (48/168)	29,5±10,47# (62/210)	90,5± 2,01 * (228/252)
	R	51,4±7,41 (86/168)	40±8,3# (84/210)	6±24,59* (15/252)
Amoxicillin	S	23,2±13,79 (39/168)	4,3±32,04* (9/210)	3,5±32,14* (9/252)
	MR	32,1±11,17 (53/168)	36,7±8,91# (77/210)	26,6±10,28# (67/252)
	R	44,7±8,34 (76/168)	59±5,65* (124/210)	70±4,06* (176/252)
Cefuroxime	S	63,7± 5,73 (107/168)	56,2± 5,99# (118/210)	53,6± 5,76* (135/252)
	MR	14,2±18,58 (24/168)	34,8±9,29* (73/210)	27,4±10,07* (69/252)
	R	22,1±14,27 (37/168)	9±21,49* (19/210)	19±12,75# (48/252)
Ceftazidime	S	89,3±2,63 (150/168)	54,8± 6,16* (115/210)	77,4±3,34* (195/252)
	MR	6,6±28,65 (11/168)	38,1±8,64* (80/210)	8,3±20,52# (21/252)
	R	2,1±48,56 (4/168)	7,1±24,44# (15/210)	14,3±15,15* (36/252)
Ciprofloxacin	S	88,1±2,79 (148/168)	78,6 ±3,54# (165/210)	83±2,81# (209/252)
	MR	9,8±23,37 (16/168)	15,7±15,7# (33/210)	6±24,59# (15/252)
	R	2,1±48,56 (4/168)	5,7±27,54# (12/210)	11±17,5* (28/252)
Furazidine K	S	84,5±3,24 (142/168)	66,7±4,79* (140/210)	85,7±2,53# (216/252)
	MR	10,7±21,89 (18/168)	27,6±10,97* (58/210)	6,3±23,76# (16/252)
	R	4,8±33,91 (8/168)	5,7±27,54# (12/210)	8±21,07# (20/252)

Notes. S — sensitive strains, MR — moderately resistant, R — resistant, CI — confidence interval, p — statistical significance according to Pearson's chi-square test, compared to Group 1, * — $p < 0.05$, # — $p < 0.05$.

THE AIM

The aim was to study the prevalence of antibiotic resistance among children with UTI over the past 10 years, with an assessment of the sensitivity of *E. Coli* to common antibiotics in dynamics.

MATERIALS AND METHODS

The study involved 1,044 children with UTI aged from 1 month to 18 years, who were treated in the Paediatric Nephrology Unit of Kyiv City Children's Clinical Hospital No. 1 in 2009, 2014 and 2019 (interval up to 5 years).

According to the design, the study included 3 comparison groups: Group 1 — children of the 2009 follow-up year ($n = 337$), Group 2 — of 2014 ($n = 328$) and Group 3 — of 2019 ($n = 379$). The examination of patients and the management of the obtained data were consistent with the provisions of the Declaration of Helsinki of Human Rights.

Diagnosis of diseases was performed according to the unified clinical protocol No. 627 of the MOH of Ukraine of November 3, 2008 "On approval of the treatment protocol for children with urinary tract infections and

Table II. Dynamics of *E. coli* resistance in patients with UTI for 2009 - 2019

Strains <i>E.coli</i>	Years		
	2009 % ± 95% CI, (abs.)	2014 % ± 95% CI, (abs.)	2019 % ± 95% CI, (abs.)
Resistant	51,2±7,41 (86/168)	59±5,65# (124/210)	70±4,06* (176/252)
Monoresistant	25±13,13 (42/168)	32,4±9,8* (68/210)	42±7,26* (106/252)
Multiresistant	26,2±12,73 (44/168)	26,6±11,24# (56/210)	28±9,97# (70/252)

Notes. p — statistical significance according to Pearson's chi-square test, compared to Group 1, * — $p < 0.05$, # — $p < 0.05$.

tubulointerstitial nephritis". The generally accepted diagnostic methods for nephrological patients were applied. Among them: complete blood count and urinalysis, liver and renal tests with calculation of glomerular filtration rate (GFR), proteinogram, measurement of C-reactive protein, Nechiporenko test and Zimnitsky test, bacterial urine culture to determine the pathogen and its sensitivity using the disc dispersion method (DDM), ultrasound of the kidneys and bladder, voiding cystourethrography and excretory urography, if necessary.

Assessment of the distribution of the studied parameters using Pearson's chi-squared test confirmed the normal distribution. Statistical hypotheses were tested using the chi-square goodness of fit test. For fractional values, the 95 % confidence interval (CI) of the parameter distribution was calculated. The absolute and relative probability frequencies were calculated by the odds ratio (OR) with the calculation of the standard error under condition of 95 % CI. The threshold value for statistical significance was set at ≤ 0.05 [11].

Mathematical and statistical calculations were obtained using Microsoft Excel programs (Microsoft Office 2013 Professional Plus, license agreement (EULAIID:O15_RTM_VL.1_RTM_RU) and STATISTICA 13.0 (StatSoftInc., Serial No. ZZS9990000099100363DEMO-L).

RESULTS

The leading uropathogen in all study groups was *E. coli* with a tendency to increase its specific gravity every 5 years. In Group 1 of patients (2009), the percentage of *E. Coli* was 47 % (158/337), in Group 2 (2014) — 64 % (210/328) and in Group 3 (2019) — 66.5 % (252/379).

The dynamics of changes in the sensitivity of *Escherichia coli* to a common range of antibiotics over the past 10 years with the statistical forecast of the distribution of parameters is shown in Table I. Among the studied classes of antibiotics there are representatives of β -lactams (ampicillin, amoxicillin, cefuroxime, ceftazidime, ciprofloxacin) and representative of nitrofurans (furazidin K).

As can be seen from Table I, in 2019, the sensitivity of *E. Coli* (in percentage of cases) to ampicillin and amoxicillin was equally low and amounted to 3.5 ± 32.14 (9/252), to cefuroxime — 53.6 ± 5.76 (135/252), to ceftazidime — 77.4 ± 3.34 (195/252), to ciprofloxacin — 83 ± 2.81 (209/252) and to furazidin K — 85.7 ± 2.53 (216/252). In turn, resistance values were as follows: to ampicillin —

6 ± 24.5 (15/252), to amoxicillin — 70 ± 4.06 (176/252), to cefuroxime — 19 ± 12.75 (48/252), to ceftazidime — 14.3 ± 15.15 (36/252) and to furazidin K — 8 ± 21.07 (20/252). Moderately resistant strains to ampicillin — 90.5 ± 2.01 (228/252), to amoxicillin — 26.6 ± 10.28 (67/252), to cefuroxime — 27.4 ± 10.07 (69/252), to ceftazidime — 8.3 ± 20.52 (21/252), to ciprofloxacin — 6 ± 24.59 (15/252), to furazidin K — 6.3 ± 23.76 (16/252) were found.

Assessment of the prevalence of resistant *Escherichia coli* with the calculation of the percentage of monoresistant and multiresistant strains and the dynamics of changes every 5 years is shown in Table II.

The data in Table II showed that the overall level of resistant *E. Coli* strains at 95 % CI in 2009 was 51.2 ± 7.41 (86/168), in 2014 — 59 ± 5.65 (124/210) and in 2019 — 70 ± 4.06 (176/252). Moreover, in 2019, the monoresistant conditions in the total sample of UTI patients were 42 ± 7.26 (106/252) cases, multiresistant — 28 ± 9.97 (70/252).

The results of assessing the relative risk (RR) of resistance formation in patients with UTI during the indicated follow-up periods and its mathematical prognosis for the next 10 years are shown in Fig. 1.

As can be seen from the graph, the relative risk of antibiotic resistance in 2009 was $RR = 0.727 \pm 0.209$ [0.483; 1.095], in 2014 — $RR = 1.375 \pm 0.209$ [0.913/2.063], $p > 0.05$ and in 2019 — $RR = 2.208 \pm 0.207$ [1.473;3.310], $p < 0.05$. According to the equation of the trend line, the relative risk of *E. Coli* resistance in 2024 will be $RR = 0.7405^4 - 0.0443 = 2.918$, and in 2029 — $RR = 3.658$ with a high statistical significance ($R^2 = 0.9948$).

DISCUSSION

The international community of nephrologists, urologists, infectious disease specialists (IDSA, EAU, AUA) identified the level of antibiotic resistance in the range of 10-20 % of strains, within which the empirical prescription of an antibacterial drug is possible [12]. If the culture test results are available, only the drugs with the sensitivity of microorganisms to these drugs should be prescribed [13]. The use of drugs with moderate resistance of the strains is also clinically justified, since it may not lead to patient recovery but may contribute to the recurrence or persistence of infection, the formation of resistance of microorganisms and other complications [14].

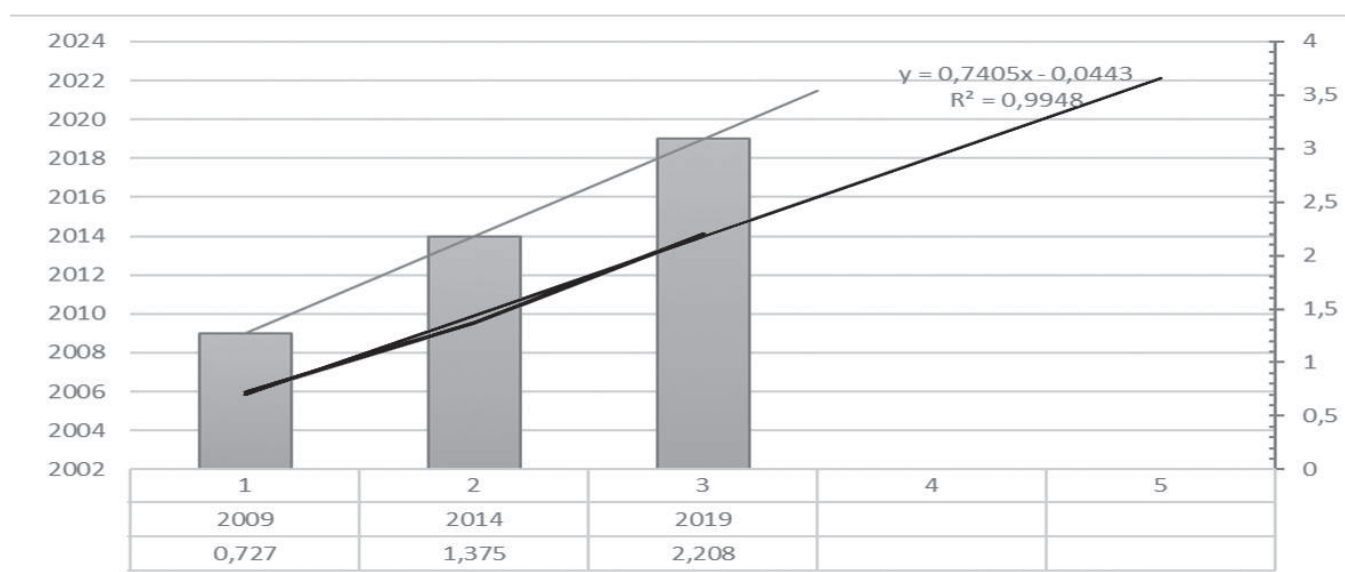


Fig. 1. Assessment and prognosis of the relative risk (RR) of antibiotic resistance in children with UTI

Notes. $y = 0.7405x - 0.0443$ is the equation of the trend line to calculate the prognostic value in the following periods, where x is the number of the period; R^2 is coefficient of determination.

The results of our study showed that neither ampicillin nor amoxicillin can be the drugs of empirical choice in the treatment of UTI in children, which coincided with the studies of other authors [15]. But we also noted that cefuroxime cannot be a first-line drug without a culture test, since only every second child confirmed sensitivity to it (53.6 ± 5.76 (135/252)), while the level of overall resistance (R strains + MR strains) was 46.4 ± 6.64 (117/252). Relatively high sensitivity was shown for ceftazidime and ciprofloxacin in 2019 (77.4 ± 3.34 (195/252) and 83 ± 2.81 (209/252), respectively), while resistance to them grew quite rapidly — almost doubled in the last 5 years. Thus, overall resistance (R + MR) in 2019 for ceftazidime was 22.6 ± 11.44 (57/252), for ciprofloxacin — 17.0 ± 13.64 (43/252). A high sensitivity of 85.7 ± 2.53 (216/252) was determined for furazidin K as the representative of nitrofurans. With the best value of the overall resistance of strains in 2019 — 14.3 ± 15.15 (36/252) and the relatively slow formation of resistance. The value increase by 1.7 times over the past 10 years.

We found that the resistance level of *E. Coli* among children with UTIs has especially grown over the past 5 years, and compared to the 10-year period, the number of resistant strains has increased by 20 %. The percentage of multiresistant strains also tended to increase so that in 2019 almost every second child with resistance had multiresistant strains — 40 ± 9.12 (70/176). We statistically evaluated and determined the current adverse trends of ABR according to the relative risk analysis. In 2019 it increased by 1.6 times compared to 2014 ($RR_{2019} = 2.208 \pm 0.207$ [1.473;3.310], $p < 0.05$ vs $RR_{2014} = 1.375 \pm 0.209$ [0.913;2.063]) and by 3 times compared to 2009 ($RR_{2009} = 0.727 \pm 0.209$ [0.483;1.095]). The mathematical equation of the trend line allowed us to determine the prognosis for ABR for the next 5-10 years if the existing diagnostic and therapeutic tactics of UTI are maintained. It was

found that the relative risk of ABR progressively increased by 2.9-3.7 times. For comparison, $RR_{2009} = 0.727 \pm 0.209$ [0.483; 1.095] and $RR_{2029} = 3.658$ with $R^2 = 0.9948$.

The study results are important for the management of paediatric UTI, both in primary health care and for optimal clinical decisions made by highly specialized doctors. Doctors should consider the effect of any use of antibiotics on further antimicrobial resistance and avoid their inadequate use. They should also adhere to data on local sensitivity and national recommendations, when the prescription of an antibacterial agent is indicated.

CONCLUSIONS

1. The antibacterial resistance of *E. Coli* strains is common among children with UTI and has high growth dynamics.
2. The resistance level of *E. Coli* was $70 \pm 4.06\%$ (176/252), which is 11 % more compared to 2014 and 18.8 % more compared to 2009.
3. The percentage of multiresistant *E. Coli* strains tended to increase and amounted to $28 \pm 9.97\%$ (70/252) among patients with UTI and $40 \pm 9.12\%$ (70/176) in the structure of resistance in 2019.
4. The relative risk of ABR in 2019 increased by 1.6 times compared to 2014 ($RR_{2019} = 2.208 \pm 0.207$ [1.473;3.310], $p < 0.05$ vs $RR_{2014} = 1.375 \pm 0.209$ [0.913;2.063]) and by 3 times compared to 2009 ($RR_{2009} = 0.727 \pm 0.209$ [0.483;1.095]).
5. Ampicillin and amoxicillin cannot be the empirical choice in the treatment of UTI, because they show an equally low sensitivity ($3.5 \pm 32.14\%$ (9/252)).
6. Cefuroxime cannot be a first-line drug without a culture test, since only every second child confirmed sensitivity to it (53.6 ± 5.76 (135/252)).

7. Ceftazidime and ciprofloxacin showed a relatively high sensitivity level — 77.4 ± 3.34 (195/252) and 83 ± 2.81 (209/252), respectively. At the same time, the rapidly growing resistance was recorded — almost doubled over the past 5 years.
8. Furazidin K showed a high sensitivity level of $85.7 \pm 2.53\%$ (216/252), the lowest level of overall resistance of $14.3 \pm 15.15\%$ (36/252) and a slow rate of its formation.
9. The prognosis for the relative risk of ABR for the next 5-10 years is determined as an increase by 2.9-3.7 times if the existing diagnostic and therapeutic tactics of UTI are maintained.

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ORIGINAL ARTICLE

MECHANISMS OF INSOMNIA FORMATION WITH GASTROESOPHAGEAL REFLUX DISEASE, TAKING INTO ACCOUNT THE PSYCHOSOMATIC STATUS IN YOUNG PEOPLE

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ABSTRACT

The aim: The work is aimed to study the formation mechanisms of sleep disturbances with GERD based on the determination of melatonin and serotonin indicators in the blood, psychosomatic status and quality of sleep, motor-secretory function of the esophagus and stomach in young people.

Materials and methods: All 63 patients were with non-erosive GERD and insomnia. Among them, 32 with inhibitory personality type and 31 with excitable personality type. The control group is 25 people. Questionnaires were used to determine the quality of sleep, quality of life, state of the nervous system, ultrasound, stomach acidity, level of melatonin and serotonin.

Results: In patients with inhibitory personality type, depressive disorders, lower sleep quality and quality of life were more pronounced, and in patients with excitable type, increased levels of reactive and personal anxiety and pronounced motor-secretory disorders.

Conclusions: Psychosomatic disorders in GERD have a significant impact not only on the quality of sleep, quality of life, the level of melatonin and serotonin, the regulation of the secretory and motor functions of the stomach, but they are one of the links in the pathogenetic mechanism of the formation of both GERD and insomnia with it.

KEY WORDS: gastroesophageal reflux disease, insomnia, life quality, melatonin, serotonin

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the leaders among diseases of the digestive tract. Despite global distribution [1], the incidence rate in different countries varies (from 2.5 in China to 51.2 in Greece) [2]. Herewith, recent studies show that GERD increasingly affects young and able-bodied population [3]. Many studies show that, in addition to age, risk factors for the development of this disease are obesity, hiatal hernia, alcohol consumption and smoking. However, a recent large crossover study showed that a high somatization level according to SSC (somatization scale) is also a common risk factor [4]. Typical symptoms of GERD can affect performance, sleep, nature of nutrition, daily activity, which significantly reduces the patient's life quality [5]. Moreover, sleep disturbance with GERD occurs from 26 to 60% and has a proven, bi-directional communication [6]. In addition, emotional disturbances, including depression and anxiety, often accompany GERD [7]. It is known that psychological stress can cause insomnia as much as GERD.

Since GERD is diagnosed more often in the adult population, the study of this pathology in young people, in particular students, is rare [8]. On average GERD can be found among students no more than among overall population. However, since it is at this age that the pathogenetic mechanisms of this disease are laid, then with appropriate measures, both therapeutic and prophylactic, one can achieve a favorable course of this disease.

Moreover, to date, the mechanisms of the formation, course, diagnostics and treatment of sleep disturbances in young people, in particular students with GERD, taking into account the psychosomatic status, are not fully understood, which determined the purpose of our study.

THE AIM

The work is aimed to study the formation mechanisms of sleep disturbances with GERD based on the determination of melatonin and serotonin indicators in the blood, psychosomatic status and quality of sleep, motor-secretory function of the esophagus and stomach in young people.

MATERIALS AND METHODS

We examined 63 students hospitalized in the gastroenterological department of the Municipal Non-Commercial Enterprise "City Student Hospital", Kharkiv, Ukraine. All patients with non-erosive GERD were divided into two clinically homogeneous groups. The first group included 32 patients with GERD and insomnia and inhibitory personality type (15 men and 17 women) whose average age was 20.5 years old. The second group — GERD with insomnia and excitable personality type — 31 patients (15 men and 16 women) whose average age was 20.1 years old. The control group consisted of 25 healthy patients (12 men

and 13 women) whose age was 20.2 years old. All patients had a non-erosive GERD form. The case history of GERD ranged from 1 to 5 years. An average is of 3.4 years. GERD was diagnosed according to ICD-10 and the recommendations of the 2006 Montreal Consensus. The criteria for the exclusion of students from the observation groups were the presence of concomitant pathology of other organs and systems and inflated self-esteem of psychosomatic status according to any of the questionnaire scales, which, in conformity with instructions, required a psychotherapist consultation.

Prior to the study, all sick and healthy students of the control group were informed of its aims. A voluntary consent for the study was obtained. All procedures performed in the study comply with the Declaration of Helsinki.

In order to study sleep disturbances in patients of both groups, the Pittsburgh Sleep Quality Index (PSQI) was used. The following components of sleep were evaluated: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, use of sleep medication, daytime dysfunction. Each component was evaluated from 0 to 3, where 0 indicates the absence of sleep disturbances, and 3 indicates serious disturbances. The sum of the scales results varies from 0 to 21. A higher score indicates the worst sleep quality.

The presence and severity of depression were determined using the PHQ-9 questionnaire (Patient Health Questionnaire). This questionnaire consists of nine questions. Each question has four possible answers and assessment from 0 to 3, depending on the problem frequency. If the total number of scores is from 0 to 4, this means the absence of depression, from 5 to 9 – mild depression, from 10 to 14 – moderate one, from 15 to 19 – moderately severe and from 20 to 27 – severe depression.

The psychosomatic condition of patients was assessed according to the Spielberger-Khanin scale to determine personal and situational anxiety. When analyzing the results, the total indicator for each of the subscales can be in the range from 20 to 80 scores. Moreover, the higher the final indicator, the higher the level of anxiety (situational or personal). When interpreting the indicators, the following anxiety assessments are used: up to 30 scores – low, 31-44 scores – moderate; 45 and more – high.

Life quality was assessed using the unified SF-36 questionnaire, which includes assessment of the physical health scale: role-physical functioning (RP), physical functioning (PF), bodily pain (BP), general health (GH), and the mental health scale: role-emotional functioning (RF), vitality scale (VT), mental health (MH), and social functioning (SF). Indicators of each scale ranged from 0 (the lowest level) to 100 scores (full health level).

The acidity of the gastric juice was determined by intragastric pH-metry using IKZh-2 apparatus (produced in Ukraine).

Motor-evacuatory function of the esophagus and stomach was identified using an ultrasound examination conducted on ALOKA SSD-650 machine (produced in Japan) and Ultima pro-30 machine (produced in Ukraine) – in

the patient's left side and on the back; in epigastric region and projections at esophageal hiatus; fasted and 5, 10 and 1 minutes after drinking 0.5 l of liquid. The study measured the thickness of the esophagus wall, the esophagus width in the lower third, the diameter of esophageal hiatus, the presence of fluid regurgitation, and its duration.

Melatonin and serotonin in blood serum were determined by immunoenzyme method using a standard IBL set of reagents (produced in Germany). Since melatonin has daily fluctuations, blood sampling was performed at the same time of the day for all subjects (7.00 in the morning).

To process statistical data, we used Microsoft Office Excel 2007 and the statistical data processing system Statistica 6.0. Under normal distribution, Student's t-test was used; in case of deviation from the normal distribution, non-parametric methods were used. The mean value (M), Student's t-test was assessed. The differences were considered significant at $p < 0.05$. The results were presented as $M \pm m$, where M is the median and m is the standard deviation.

RESULTS

In the course of our studies, we found that in the clinic picture in most patients (76.8%) among both excitable and inhibitory ones, heartburn was the guiding symptom.

When studying the sleep quality index in GERD patients with insomnia according to the Pittsburgh Questionnaire (PSQI), the following results were revealed: subjective sleep quality in group 1 was 1.86, in group 2 – 2.47, in the control group – 0.53. The index of sleep latency in group 1 was 2.4, in group 2 – 2.7, in the control group – 0.32. The sleep duration indicator in group 1 was 0.95, in group 2 – 1.46, in the control group – 0.3. The sleep efficiency index in patients of group 1 was 1.05, in group 2 – 1.54, in the control group – 0.24. The rate of sleep disturbance in group 1 was 1.16, in group 2 – 1.52, in the control group – 0.25. None of the patients used sleeping pills, so this indicator for all was 0. The daytime dysfunction indicator in group 1 was 1.27, in group 2 – 2.01, in the control group – 0.15. The average total score for group 1 was 8.72, for group 2 – 11.7, for the control group – 1.8.

Thus, sleep disturbance was observed in patients of both groups, but insomnia ($p < 0.05$) was more pronounced in patients of group 2 as compared not only to the control group, but also to the patients of group 1.

When studying the presence and severity of depression in GERD patients with insomnia according to PHQ-9 (Patient Health Questionnaire), the following results were revealed. Moderate depression was more pronounced in patients of group 2 – 82.12% than in group 1 – 67.32%, and in the control group it was absent ($p < 0.05$). Mild depression in group 1 was 20.63%, in group 2 – 13.34%, in the control group – 3.3% ($p < 0.05$). Depression was absent in 96.7% in the control group, in group 1 – 12.05%, in group 2 – 4.54% ($p < 0.05$).

When determining the psychosomatic status in GERD patients with insomnia, as well as in people of the control group, the following results were obtained. Personal

anxiety in the excitable type is 49.1 ± 1.4 , in the inhibitory type – 36.1 ± 1.1 , and in individuals in the control group – 22.4 ± 1.2 ($p > 0.05$). Reactive anxiety in the excitable type is 46.8 ± 1.9 , in the inhibitory – 36.9 ± 1.2 , and in the control group – 21.9 ± 1.4 ($p > 0.05$).

With a comprehensive assessment of the psychosomatic status of patients, the following conclusions can be drawn: in the inhibitory type, the depression indicators come to the fore, and in the excitable one increased indicators of personal and reactive anxiety are more pronounced.

The life quality indicators for GERD patients with insomnia, as well as for individuals of the control group according to the unified SF-36 questionnaire, are as follows: physical functioning in group 1 was 43.7 ± 3.8 , in group 2 – 40.3 ± 3.9 , in the control group – 84.5 ± 4.0 ; role-physical functioning in group 1 was 27.0 ± 3.3 , in group 2 – 36.7 ± 4.0 , in the control group – 71.4 ± 3.0 ; the bodily pain in group 1 was 41.2 ± 3.6 , in group 2 – 23.1 ± 3.8 , in the control group – 76.3 ± 4.0 ; general health in group 1 was 35.5 ± 3.9 , in group 2 – 32.2 , in the control group – 74.9 ± 4.1 ; vitality in group 1 was 45.5 ± 3.2 , in group 2 – 31.5 ± 4.2 , in the control group – 80.5 ± 3.2 ; social functioning in group 1 was 45.2 ± 3.5 , in group 2 – 43.6 ± 3.3 , in the control group – 81.4 ± 4.4 ; role-emotional functioning in group 1 was 48.3 ± 3.1 , in group 2 – 31.7 ± 3.6 , in the control group – 78.6 ± 3.5 ; mental health in group 1 was 44.5 ± 3.3 , in group 2 – 30.5 ± 2.7 , in the control group – 75.4 ± 3.7 .

It was found that in patients of both groups, the life quality indicators were significantly reduced according to all scales of the SF-36 questionnaire as compared to the control group and the difference was statistically significant ($p < 0.001$). Moreover, in patients of group 2, indicators of vitality, bodily pain, role-emotional functioning and mental health were significantly ($p < 0.001$) lower than in patients of group 1. At the same time the indicator of role-physical functioning was lower ($p < 0.001$) in patients 1. The indicators of general health, physical and social functioning were lower in patients of group 2 as compared to group 1, but the difference was statistically insignificant ($p > 0.05$).

The indicators of pH-metry of the stomach body in patients of group 1 are 0.91 ± 0.25 , in group 2 – 1.34 ± 0.05 , in the control group – 1.61 ± 0.1 ; in the antrum, group 1 had 5.9 ± 0.15 , group 2 had 4.7 ± 0.17 , and control group had 7.21 ± 0.12 .

In patients with excitable type, the pH-metry level in the body was statistically significantly lower ($p < 0.05$) than in the group of patients with the prevalence of inhibitory personality traits in the psychosomatic status and in the control group. The neutralizing stomach activity in both groups was lower than that of the control group ($p < 0.05$).

When examining motor-evacuatory function of the esophagus and stomach in GERD patients with insomnia, as well as in the control group, the following results were revealed: the esophagus width in the lower third in patients of group 1 was 2.63 ± 2.8 cm., in patients of group 2 – 2.57 ± 0.27 cm., in the control group – 2.11 ± 0.1 cm.; the diameter of esophageal hiatus in patients of group 1

was 1.93 ± 0.27 cm., in patients of group 2 – 1.83 ± 0.25 cm., in the control group – 1.51 ± 0.12 cm.; the presence and duration of gastroesophageal reflux in patients of group 1 was $9 \text{ minutes} \pm 1.3$, in patients of group 2 – $7 \text{ minutes} \pm 1.3$, in the control group – $5 \text{ minutes} \pm 1.1$; the thickness of the esophagus wall in patients of group 1 was 0.46 ± 0.05 cm., in patients of group 2 – 0.40 ± 0.03 cm., in the control group – 0.31 ± 0.07 cm.

Thus, it was found that all indicators were significantly greater in patients of group 1 than in group 2 ($p < 0.05$).

When studying the melatonin and serotonin indicators in blood serum in GERD patients with insomnia, as well as in the control group, the following results were revealed: melatonin in patients of group 1 was 14.4 ± 1.5 pg/ml, in patients of group 2 – 7.5 ± 0.74 pg/ml, in the control group – 22.3 ± 1.8 pg/ml. Serotonin in patients of group 1 was 81.7 ± 5.3 pg/ml, in patients of group 2 – 118.3 ± 7.2 pg/ml, in the control group – 34.7 ± 2.7 pg/ml.

Thus, in patients with inhibitory type, we observed the lowest melatonin and the highest serotonin indicators.

DISCUSSION

Sleep disturbances with GERD can be associated with several reasons [9]. The main one is the presence of night reflux and night heartburn [10]. The following causes of sleep disturbance with GERD are the presence of anxiety and depression. Okuyama et al. show that patients with GERD have more frequent manifestations of anxiety and depression as compared to the control group [11]. These data are consonant with our study, since increased anxiety and depression were significantly higher in patients of both groups. But we have also shown the difference between these indicators in inhibitory and excitable young patients.

In a study of medical students in Iran who suffered from GERD, 47.2% of them had anxiety, 22.5% had depression, 36% had somatic symptoms, and 36% had social dysfunction [8]. In our study, the mentioned indicators are higher and this may be due to the presence of insomnia in our patients, which exacerbates the course of GERD; it has a direct influence on the indicators of psychosomatic status of patients.

The relationship between GERD and psychosomatic disorders has been shown in many studies [12]. A study by Ping Li et al. showed that a combination of several atypical GERD symptoms increases the risk of psychological disturbances [13].

It is known that patients with depression have a higher severity of various symptoms and are more likely to describe the symptoms in a negative light [11]. In our study, the patients with inhibitory type had a higher level of depressive disorders and life quality. Their sleep quality was significantly lower not only in comparison with the control group, but also with the excitable type.

Our observations showed a significant decrease in the quality of life in patients of both groups. Persistent GERD symptoms cause a decrease in life quality and social activity [14]. According to large studies, GERD patients with sleep

disturbances had lower life quality indicators associated with health as compared to GERD patients without sleep disturbances [15]. The physical component of life quality was particularly reduced [16]. At the same time, sleep disturbances significantly reduce not only the life quality but also the effectiveness of leisure and work productivity, which is also an important economic problem.

A significant number of studies determine sleep quality using the PSQI questionnaire. Sleep disturbances in this questionnaire were more common in patients with GERD than in the control group. 52% experienced sleep disturbances, with PSQI indicators of more than 5.5 [7]. Our patients in both groups had PSQI of more than 6.2.

The presence of stress in the life of students is not in doubt. At least 82% of the 2246 studied students suffered from physical or mental distress [17].

Herewith a number of studies have shown that patients who are under chronic stress and in state of anxiety may experience esophageal hypersensitivity, which is included in the pathogenesis of GERD [11].

At the same time, prolonged and intense exposure to psychological stressors leads to overload and depletion of the central nervous system, resulting in disorder of its regulatory function, which, together with functional disorders of organs and systems, creates the prerequisites for the development of psychosomatic pathology [18].

Taking into account our results [19] and the results of other studies [8], we can talk about a close relationship between psychosomatic disorders and GERD.

Stress can increase or trigger the formation of GERD. At the same time, the perception of GERD symptoms itself is enhanced much more than the actual presence of the symptoms severity. A number of studies show that stress-reducing GERD therapy reduces the perception of symptoms and reduces the effect of acid on the esophagus, which in turn confirms the interaction of the brain with the digestive tract [20].

Numerous studies show that stress changes the stomach acidity [21]. In our study, we also obtained the characteristics of acidity in patients with GERD, depending on the personality type and psychosomatic disorders.

Melatonin is a hormone that controls sleep and gastrointestinal motility. Low melatonin indicators can cause both GERD and insomnia [22,23]. In our study, patients in both groups also had low melatonin indicators. Serotonin and melatonin are in constant opposition and create a balance in the body. The motility of the digestive tract is a clear illustration of this process [24].

CONCLUSIONS

1. It was found that students with GERD have marked disturbances of sleep, quality of life, motor-secretory disorders, and psychosomatic disorders. Moreover, in patients with inhibitory type in psychosomatic status, depressive disorders, lower sleep quality, and life quality come to the fore, while in patients with excitable type – increase in the level of reactive and personal anxiety and pronounced motor-secretory disorders.

2. In patients of both groups, a significant decrease in the level of melatonin is determined, with a simultaneous increase in the level of serotonin. In patients with inhibitory personality type, these differences are more significant as compared to the group of GERD patients with excitable personality type.
3. A clear correlation was established between the degree of decrease in melatonin level, increased serotonin, sleep quality indicators, characteristics of psychosomatic disorders, life quality, and motor-secretory disorders of the esophagus and stomach.
4. The data obtained indicate that psychosomatic disorders with GERD significantly affect only the sleep quality, life quality, the level of melatonin and serotonin, the regulation of the secretory and motor functions of the stomach, but they are also one of the links of the pathogenetic mechanism for the formation of both GERD and insomnia.

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ORIGINAL ARTICLE

PROCALCITONIN IN EARLY PREDICTION OF ACUTE SEVERE PANCREATITIS

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ABSTRACT

The aim of the study was to analyze the prognostic potential of procalcitonin in acute pancreatitis complicated by ascites-peritonitis.

Materials and methods: The study analyzed the results of a comprehensive examination and treatment of 18 patients with acute pancreatitis complicated by enzymatic ascites-peritonitis, including 13 patients who were treated in the surgical department of KP "Poltava Regional Clinical Hospital. MV Sklifosovsky POR", and 5 patients of other emergency hospitals in Poltava, in the period from 2017 to 2019. In addition to standard screening methods, these patients were additionally tested for procalcitonin to predict an adverse course in the early period.

Results: To assess the relationship between the presence of elevated procalcitonin levels at the time of hospitalization of 0.5 ng / ml and above and unsatisfactory treatment results, differences were assessed using an accurate Fisher test. When comparing differences in the development of infectious complications in the dynamics of the disease in patients of the study group depending on the presence of elevated concentrations of procalcitonin or its absence at the time of hospitalization, a significant difference was found ($p < 0.05$).

Conclusions: In our opinion, the use of procalcitonin as a predictor of infectious complications in the dynamics of the disease will determine the category of patients in whom reducing the risk of flora translocation through the use of early oral antibiotic prophylaxis and parenteral drugs tropic to pancreatic tissue may reduce the incidence of purulent complications. In another category of patients, antibacterial therapy is not advisable due to the low risk of purulent-septic complications.

KEY WORDS: acute pancreatitis, procalcitonin, ascites-peritonitis, severity of the condition

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INTRODUCTION

Acute severe pancreatitis manifests itself as a sudden and important occurrence with a rapid deterioration of hopes [1]. Ascites-peritonitis in acute pancreatitis are quite common results of the early period of action [2]. During the first week of acute severe pancreatitis, in most cases death occurs as a result of multiple organ failure, in the following weeks, the infection plays a more important role [3]. Tang J.C.F. [3] recommends identifying patients with acute pancreatitis who are most in need of aggressive medical care, differentiating the severity of their disease.

Severity prediction is a valuable tool in the clinic, but in order to increase clinical benefit, it must anticipate real severity factors – (peri) pancreatic necrosis and / or organ failure [4]. Identification of early markers is important because there is a concern, especially among resuscitators, that patients are often hospitalized in the intensive care unit too late [5].

In the article "Predicting the prognosis of acute pancreatitis" C. Forsmark, D. Yadav [6] point to the need to identify the best biological markers of the main factors that determine the outcome – organ failure and pancreatic necrosis, as well as factors related to with the development of severe systemic inflammatory response to pancreatic necrosis as well as factors of the patient's body associated with development severe systemic inflammatory response to pancreatic necrosis. This approach can help in understanding the mechanisms of severe disease, identifying patients at risk shortly after or before damage to pancreatic tissue, and developing treatments to reduce the severity of the disease [6].

Procalcitonin is an inactive 116-amino acid propeptide of the biologically active hormone calcitonin, which, as first described, is characterized by significant concentrations in patients with bacterial and fungal infections [7].

According to the recommendations of WSES 2019, the level of procalcitonin 3.8 ng / ml and above 96 hours after the onset of the disease indicates pancreatic necrosis with a sensitivity of 93% and a specificity of 79% [8,9].

Antibiotics are always recommended for the treatment of infected severe acute pancreatitis. However, the diagnosis of infected pancreatitis is difficult because the clinical picture is no different from the inflammatory reaction caused by pancreatitis itself. The timing of infection in pancreatic necrosis is variable and unpredictable, although their peak is observed mainly from the second to the fourth week from the onset of the disease. Clinical signs are characterized by high sensitivity, but insufficient specificity [10,11].

Procalcitonin is a promising serological marker of pancreatic infection, especially pancreatic infection in combination with organ failure [12]. Specificity for procalcitonin in the meta-analysis ranging from 83 to 91% [13,14].

THE AIM

The aim of the study was to analyze the prognostic potential of procalcitonin in acute pancreatitis complicated by ascites-peritonitis.

Table I. Procalcitonin level at the time of hospitalization and after 72 hours.

Procalcitonin level (ng / ml)	at the time of hospitalization		after 72 hours	
	Abs.	%	Abs.	%
<0,1	2	11	1	6
0,1-0,4	9	50	11	61
0,5-2	4	22	4	22
2,1-10	3	17	2	11
>10	0	0	0	0

Table II. Differences in the presence of unsatisfactory treatment results depending on the presence or absence of elevated concentrations of procalcitonin at the time of hospitalization were determined by Fisher's exact test

	Elevated procalcitonin concentration of 0.5 ng / ml and above				p
	Missing		Available		
	Abs.	%	Abs.	%	
Death	1	6	3	17	0,093
Organ dysfunction after 72 hours	3	17	5	28	0,066
Development of infectious complications	1	6	6	33	0,005

MATERIALS AND METHODS

The study analyzed the results of a comprehensive examination and treatment of 18 patients with acute pancreatitis complicated by enzymatic ascites-peritonitis, including 13 patients who were treated in the surgical department of KP "Poltava Regional Clinical Hospital. MV Sklifosovsky POR", and 5 patients of other emergency hospitals in Poltava, in the period from 2017 to 2019.

In addition to standard screening methods, these patients were additionally tested for procalcitonin to predict an adverse course in the early period.

Statistical analysis of the study materials was performed using the program "STATISTICA 10.0" (StatSoft, Inc., USA) by methods of descriptive statistics with the calculation in the studied groups of quality indicators in the form of frequencies and their percentages.

The statistical significance of differences was determined by a nonparametric method between the indicators of independent groups using Fisher's exact criterion. Differences at $p < 0.05$ were considered statistically significant for all types of analysis.

This set of statistical methods for evaluating the results of treatment allowed to obtain evidence-based results.

RESULTS AND DISCUSSION

We studied the level of procalcitonin during hospitalization and after 72 hours in patients with acute pancreatitis complicated by ascites-peritonitis. Enzyme-linked immunosorbent assays were performed on the basis of the Research

Institute of Genetic and Immunological Foundations of Pathology and Pharmacogenetics "UMSA", Poltava. The level of procalcitonin was determined using a test system: "A set of reagents for enzyme-linked immunosorbent assay of procalcitonin concentration in human serum (plasma)" Procalcitonin-IFA-BEST"; VECTOR-BEST, Russia".

In patients of the study group, the values of procalcitonin at the time of hospitalization and after 72 h were distributed as follows (Table I).

To assess the relationship between the presence of elevated procalcitonin levels at the time of hospitalization of 0.5 ng / ml and above and unsatisfactory treatment results, differences were assessed using an accurate Fisher test.

The following indicators were selected as criteria and target points for the analysis of disease dynamics:

1. Organ dysfunction after 72 hours
2. Development of infectious complications in the dynamics of the disease.
3. Mortality (Table II).

When comparing differences in the development of long-term organ dysfunction and mortality in patients of the study group depending on the presence of elevated concentrations of procalcitonin or its absence at the time of hospitalization, no significant difference was found ($p > 0.05$).

When comparing differences in the development of infectious complications in the dynamics of the disease in patients of the study group depending on the presence of elevated concentrations of procalcitonin or its absence at the time of hospitalization, a significant difference was found ($p < 0.05$).

According to the recommendations of WSES 2019, the level of procalcitonin 3.8 ng / ml and above 96 hours after the onset of the disease indicates pancreatic necrosis with a sensitivity of 93% and a specificity of 79% [8,9]. At the time of hospitalization, at the time of hospitalization and after 72 hours in patients of the study group, the concentration of procalcitonin did not exceed 3.8 ng / ml. However, procalcitonin is recognized as an effective predictor of the severity of acute pancreatitis and the risk of developing infected pancreatitis [7]. A number of studies demonstrate the effectiveness of determining the serum concentration of procalcitonin in predicting the risk of infected pancreatic necrosis [13,15–17]. Therefore, in predicting the course of pancreatitis, the following indicators are important:

- 0.5 ng / ml during hospitalization and maintaining the level of increase in the dynamics;
- above 2.0 ng / ml is traditionally interpreted as a high risk of need for surgery for infectious complications;
- ng / ml 96 hours after the onset of the disease indicates infected pancreatic necrosis.

Values of procalcitonin concentration of 0.5 ng / ml and more are interpreted as pathological and can be used to predict the severity of acute pancreatitis and the development of infected pancreatic necrosis in the dynamics of the disease [13].

The obtained results suggest that the change in treatment tactics in patients with acute severe pancreatitis complicated by ascites-peritonitis who have elevated procalci-

tonin levels (> 0.5 ng / ml) at the time of hospitalization and maintenance / increase of its level in the dynamics at re-examination after 72 h may help reduce the risk of purulent-septic complications.

CONCLUSIONS

In our opinion, the use of procalcitonin as a predictor of infectious complications in the dynamics of the disease will determine the category of patients whose risk of translocation of flora is reduced by the use of early oral antibiotic prophylaxis and parenteral drugs tropic to pancreatic tissues may reduce the incidence of purulent complications. In another category of patients, antibacterial therapy is not advisable due to the low risk of purulent-septic complications.

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ORIGINAL ARTICLE

CARBOHYDRATE AND LIPID METABOLIC PROFILES OF TUBERCULOSIS PATIENTS WITH BILATERAL PULMONARY LESIONS AND MYCOBACTERIA EXCRETION

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ABSTRACT

The aim: To assess carbohydrate and lipid metabolic profiles of tuberculosis patients with bilateral injuries of the lungs and mycobacteria excretion.

Materials and methods: Seventy two newly diagnosed pulmonary TB patients were examined. Group I – 17 newly diagnosed TB patients who had unilateral pulmonary lesions and had no mycobacteria excretion. Group II – 55 newly diagnosed TB patients who had bilateral pulmonary lesions and mycobacteria excretion. The control group included 20 healthy persons. Fasting insulin level, indices of lipidogram were measured, oral glucose tolerance test was performed. Statistical processing of the obtained results was carried out by analyzing the contingency tables using the StatisticaBasicAcademic 13 for Windows software package.

Results: Tuberculosis patients develop insulin resistance – condition that is a precursor to developing type 2 diabetes and metabolic disorder of lipid exchange – dyslipidemia. Patients with bilateral pulmonary lesions and mycobacteria excretion have the most pronounced disorders of carbohydrate and lipid metabolism compared to patients with limited lesions of the lungs.

Conclusions: We suppose that mycobacteria excretion and bilateral lesions of lungs may be the markers of the degree of carbohydrate and lipid metabolism disorders in patients with pulmonary tuberculosis.

KEY WORDS: pulmonary tuberculosis, mycobacteria excretion, glucose metabolism, lipidogram

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INTRODUCTION

Diabetes mellitus (DM) leads to increased susceptibility to tuberculosis (TB) via multiple mechanisms. The mechanisms include those directly related to hyperglycaemia and cellular insulinopenia, as well as indirect effects on macrophage and lymphocyte function. DM is known to affect chemotaxis, phagocytosis, activation, and antigen presentation by phagocytes in response to *M. tuberculosis* [1]. But the link between DM and TB is bidirectional. TB can lead to impaired glucose tolerance (IGT) and new onset DM [2]. Dysglycemia in TB patients might be the result of stress response to infection, though dedicated studies to assess whether TB increases the risk of diabetes are still few.

THE AIM

The study was performed to assess changes in carbohydrate and lipid metabolic profiles in tuberculosis patients with bilateral lesions of the lungs and mycobacteria excretion.

MATERIALS AND METHODS

We examined 72 newly diagnosed pulmonary TB patients, who were treated in Kharkiv Regional TB Dispensary No.

1 from 2016 to 2017. Those patients who had HIV/TB co-infection, TB/DM comorbidity and body mass index (BMI) >25 were excluded from the study. Depending on the mycobacteria excretion, which was confirmed by sputum microscopy and sputum culture test, and the extent of pathological changes in the lungs on X-ray, patients were divided into groups. The control group included 20 healthy persons, which were correlated with the comparison groups by gender and age.

Additionally we performed oral glucose tolerance test (OGTT) and measured fasting insulin level. Also, indices of lipidogram were measured: total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low density lipoproteins (VLDL), triglycerides and atherogenicity index (AI). BMI and HOMA-IR were calculated.

Statistical processing of the obtained results was carried out by analyzing the contingency tables using the StatisticaBasicAcademic 13 for Windows software package. We used the median (Me) interquartile range (Lower – lower quartile, Upper – upper quartile) and sample size (min – minimum, max – maximum value). The difference between groups was determined by non-parametric statistics using the Kolmogorov-Smirnov test and Mann-Whitney test

Table 1. Signs and symptoms on admission

Signs and symptoms	Group I (unilateral lesions, MTB-) n=17	Group II (bilateral lesions, MTB+) n= 55
Fatigue	11 (64.7 %)	31 (56.4 %)
Loss of appetite	3 (17.6 %)	5 (9.1 %)
Fever	5 (29.4 %)	38 (69.31 %)
Night sweats	5 (29.4 %)	20 (36.4 %)
Coughing that lasts three or more weeks	5 (29.4 %)	30 (54.5 %)
Chest pain, or pain with breathing or coughing	0	3 (5.5 %)
Unintentional weight loss	0	8 (14.5 %)

criteria (MWC). To study the independent variables, we used the non-parametric Kruskal-Wallis test criteria and the median test.

The work was performed according to the requirements for researches with the participation of people: Statute of the Ukrainian Association for Bioethics and the GCP norms (1992), requirements and norms of ICH GLP (2002), typical ethics provisions of the Ministry of Public Health of Ukraine 66 dated February 13, 2006.

RESULTS AND DISCUSSION

Group I consisted of 17 newly diagnosed TB patients who had unilateral pulmonary lesions and had no mycobacteria excretion (23.6 %). Group II included 55 newly diagnosed TB patients who had bilateral pulmonary lesions and mycobacteria excretion (76.4 %). Age and sex distribution were nearly the same in both groups. Age ranged from 19 to 58. Men prevailed in both groups: 11 (64.7 %) and 44 (80 %) respectively. The overwhelming majority of Group I patients were hospitalized to the TB hospital in satisfactory condition. Clinical course of the Group II patients was aggravated. They were often complained of signs and symptoms typical for active pulmonary tuberculosis. More detailed information presented in the Table 1.

Infiltrative TB was diagnosed in sixteen patients (94.1%) from group I and in fifty three patients (96.4%). One patient from group I (5.9%) was diagnosed with miliary TB and two patients (3.8%) from group II had fibrous-cavernous TB.

In group I patients pulmonary lesions on X-Ray, mainly were localized within one lobe (88.2%), cavitation had three patients (17.6%). All patients from group II had lung cavitation and the multiple decay cavities of various sizes were prevailed – 81.8% (45 patients).

BMI was within normal ranges in most patients of both groups, though 10 patients from Group II (18.2%) had expressed underweight (BMI < 16.0).

When comparing medians of the carbohydrate profile indicators of TB patients and healthy controls (Table 2.), we found that the median of fasting glucose levels were

within normal ranges (3.5 -5.3 mmol/L) in both groups, though it was higher among TB patients (5.1 mmol/L vs 3.9 mmol/L). But these differences were not significant ($p>0.01$, CMW). Oral glucose tolerance test revealed that the median of postprandial glucose levels did not have significant differences (4.3 mmol/L vs 4.0 mmol/L). We found statistically significant difference ($p<0.01$, CMW) in median of fasting insulin levels of TB patients and healthy controls (16.15 mcU/ml vs. 4.8 mcU/ml). HOMA-IR also was statistically significantly higher ($p<0.01$, CMW) among TB patients (5.8 vs. 0.8). Medians of lipid metabolism indices of TB patients and healthy controls were within normal ranges (Table 2.), except atherogenicity index which was significantly higher ($p<0.01$, CMW) among the group of patients (2.8 vs 1.5). This changes were associated with elevated total cholesterol level among TB patients compared to healthy controls (4.3 vs 3.8mmol/L) and decreased level of HDL (1.0 vs 1.3 mmol/L). At the same time, we found an increase in median level of LDL in the group of TB patients (2.4 vs 1.7 mmol/L).

When comparing the results obtained in groups of patients, we found that among patients of group II, who had bilateral pulmonary lesions and mycobacteria excretion, pronounced metabolic changes were revealed. The median of fasting glucose levels was significantly higher ($p<0.01$, CMW) among group II patients (5.4 mmol/L vs 4.2 mmol/L). Postprandial glucose levels during oral glucose tolerance test did not have significant differences but the median meaning was also higher among group II patients (5.1 mmol/L vs 4.2 mmol/L). The median of fasting insulin levels was significantly higher ($p<0.01$, CMW) in group II patients (26.2 mcU/ml vs. 10.8 mcU/ml) and it is even exceeded normal ranges (2 mcU/ml – 25 mcU/ml). HOMA-IR also was significantly higher ($p<0.01$, CMW) among patients who had bilateral pulmonary lesions and mycobacteria excretion (6.8 vs. 2.6).

The median of total cholesterol level exceeded the norm in group II and it was significantly higher compering to the results of group I patients (5.6 vs 4.0 mmol/L). Level of HDL was within normal ranges and had no significant differences between groups (1.2 vs 1.0 mmol/L). The median level of LDL in both groups also were within normal ranges but the results of group II were significantly higher comparing to the group I (3.2 vs 2.1. mmol/L). Atherogenicity index excided the norm in both groups and was higher in group II patients (3.2 vs 2.8) ($p<0.01$, CMW).

Studies show that many people with type 2 diabetes also suffer from interstitial lung disease. The pro-inflammatory, proliferative, and oxidative properties of hyperglycemia have been shown to have an important role in affecting pulmonary vasculature, airways, and lung parenchyma [3-5]. But inflammatory lung diseases also can be a reason of glucose metabolism disorders. Findings of Cyphert TJ and others show that targeted induction of low-grade inflammation in the lung airway epithelium that does not result in systemic hypoxemia triggers systemic insulin resistance [6]. The same findings were revealed during our study, patients with newly diagnose pulmonary TB had

Table II. Glucose and lipid metabolism indices in pulmonary TB patients and control group

Parameter, (units)	mean	median	min	max	Lower	upper	Stn.dev.
pulmonary TB patients							
Fasting blood glucose level, (mmol/L)	5.3	5.1	2.4	10.5	4.7	5.6	1.7
2-hour glucose level, (mmol/L)	4.92	4.3	2.8	9.8	3.6	6.2	1.7
Fasting blood insulin level, (mcU/ml)	18.2	16.5	1.8	53.3	8.2	26.1	11.6
HOMA –IR	5.9	5.8	1.9	26.9	3.9	7.3	2.2
Total cholesterol, (mmol/L)	4.5	4.3	3.8	5.1	4.1	4.4	0.4
Triglycerides, (mmol/L)	1.4	1.3	1.2	1.4	1.3	1.3	0.1
HDL, (mmol/L)	1.2	1.0	0.8	1.6	1	1.3	0.2
LDL, (mmol/L)	2.6	2.4	2.1	3.3	2.2	2.9	0.4
VLDL, (mmol/L)	0.6	0.6	0.5	0.7	0.6	0.6	0.1
AI	2.9	2.8	1.9	3.5	2.7	3.1	0.5
control group							
Fasting blood glucose level, (mmol/L)	4.1	3.9	3.0	5.8	3.5	4.8	0.9
2-hour glucose level, (mmol/L)	3.9	4	2.8	5.7	3.2	4.5	0.9
Fasting blood insulin level, (mcU/ml)	4.7	4.8	2.6	6.1	4.1	5.2	1
HOMA –IR	0.9	0.8	0.5	1.5	0.7	0.9	0.2
Total cholesterol, (mmol/L)	3.8	3.8	2.9	4.7	3.4	4.2	0.5
Triglycerides, (mmol/L)	1.3	1.3	1.2	1.5	1.3	1.4	0.1
HDL, (mmol/L)	1.5	1.3	1.2	1.7	1.4	1.6	0.2
LDL, (mmol/L)	1.7	1.7	0.8	2.4	1.4	2.2	0.5
VLDL, (mmol/L)	0.6	0.6	0.5	0.6	0.6	0.6	0.1
AI	1.6	1.5	0.8	2.3	1.4	1.9	0.5

increased indices of fasting insulin levels and HOMA-IR. Moreover, we revealed significant increase both of these indices in TB patients who had bilateral pulmonary lesions and mycobacteria excretion, comparing to those patients who had limited lesions in their lungs and had no mycobacteria excretion.

Insulin resistance lead to increased synthesis and secretion of triglycerides, very low-density lipoprotein (VLDL) particles by the liver, an increase in plasma triglycerides, enrichment of plasma HDL particles by triglycerides and more active catabolism of triglyceride-rich HDL-C [7]. Unlike these research data we did not find an increase in plasma triglycerides level in pulmonary TB patients. Instead we revealed signs of dyslipidemia: an increase in total cholesterol level due to an increase in the LDL fraction and a decrease in HDL and increase in atherogenicity index. These changes were also more pronounced among patients with bilateral pulmonary lesions and mycobacteria excretion.

CONCLUSIONS

According to our results, patients with newly diagnosed pulmonary tuberculosis develop insulin resistance – con-

dition that is a precursor to developing type 2 diabetes and metabolic disorder of lipid exchange – dyslipidemia.

Patients with bilateral pulmonary lesions and mycobacteria excretion have the most pronounced disorders of carbohydrate and lipid metabolism compared to patients with limited lesions of the lungs.

We suppose that mycobacteria excretion and bilateral lesions of lung tissue may be the markers of the degree of carbohydrate metabolism disorders in patients with pulmonary tuberculosis.

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ORIGINAL ARTICLE

APPLICATION OF VITAMIN D IN DIFFERENT DOSAGE TO TREAT CHILDREN WITH ALLERGIC DISEASES

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ABSTRACT

The aim: To study the dynamics of the level of 25(OH)D, IL-4, IL-10, and IgG in the blood serum of children with allergic diseases and to study the clinical effect of vitamin D₃ administration in different dosage in this category of patients.

Materials and methods: 153 children aged 3–16 with such allergic diseases as bronchial asthma, atopic dermatitis and allergic rhinitis have been examined. The level of 25(OH)D was determined using the electrochemiluminescence method, while the levels of IL-4, IL-10 and IgG were assessed using enzyme-linked immunoassay.

Results: In the contrasting of the initial level of 25(OH)D in the blood serum of patients after administration of 2,000 IU of vitamin D₃ over 2 months, after summer and after treatment with cholecalciferol in higher doses (4,000–5,000 IU) over 2 months, significant difference was established between the indicators by the Friedman criterion ($\chi^2 = 41.211$; $P < 0.05$). In the similar contrasting of IL-4 indicators, a significant difference between them was traced ($P < 0.05$) in the period of acute disease as well as the downward tendency in the period of remission. In the similar contrasting of IL-10 indicators, a significant difference between them was traced ($P < 0.05$) in the acute period and in the period of disease remission. In the similar contrasting of IgG indicators, a downward tendency was traced in the period of acute disease and significant decrease ($P < 0.05$) – in the period of disease remission. In the contrasting of 25(OH)D and IL-4, IL-10 figures a strong reverse correlation relationship was traced. The therapeutic effect of the administration of vitamin D₃ medication in different doses in children with allergic diseases was traced.

Conclusions: The data obtained shows that in the treatment of children with bronchial asthma, allergic rhinitis and atopic dermatitis the complex therapy should include vitamin D₃ medications in different doses within a long-term course of treatment.

KEY WORDS: children, allergic diseases, treatment, vitamin D

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INTRODUCTION

Over the last decade the ideas about the normal concentration of vitamin D in human blood has changed. In 2011 the new classification of vitamin D-status in human organism was introduced, under which vitamin D deficit shall be established at the level of 25(OH)D in the blood serum below 50 nmole/l, or below 20 ng/ml, vitamin D deficiency shall be diagnosed at the level of 25(OH)D between 50–75 nmole/l, or 21–30 ng/ml, while the level of 25(OH)D from 75 to 150 nmole/l, or 31–85 ng/ml shall be considered to be within the norm [1]. According to the results of some scientific research, the best and most desirable level of 25(OH)D in the blood serum of human body shall be considered that of 36–48 ng/ml [2].

According to the results of large-scale studies, it has been established that hypovitaminosis D is characteristic of many residents of different countries of the world and is a widespread problem among adults and children [3, 4, 5].

In particular, there are numerous scientific researches confirming involvement of vitamin D in the immune processes of human body [6, 7]. It has been proven that hypovitaminosis D constitutes one of the factors causing not just development of the pathology of the musculo-skeletal system, but many diseases of other bodies and systems [8, 9, 10, 11].

It is known that development of such diseases as bronchial asthma, atopic dermatitis and allergic rhinitis takes place during pathological processes occurring in the immune system. According to the conclusions of scientific researches, it has been established that the key role in the allergic inflammation is played by IL-4, IL-5, IL-10, and IL-13 [12]. It is considered that IL-4 is the main cytokine ensuring switching of B-lymphocytes to IgE synthesis, while IL-10 is its synergist. It is also known that IgG is the main class of antibodies, actively involved in the organism protection against bacteria, viruses and toxins, its intensified release by plasma cells starts only after repeated interaction with antigen. IgG includes several subpopulations: IgG1, IgG2, IgG3, and IgG4, and the latter, in its turn, participates in the allergic inflammation processes [13]. Due to this, in laboratory study of patients with allergic diseases attention should be paid to the level of the above cytokines and immunoglobulin in the patient blood.

Currently, many countries of the world regulate taking of vitamin D medications by people of different age categories. In 2012 the scientific and practical conference in Warsaw, where scientists from different countries of the world participated, approved recommended doses of vitamin D medications for the residents of Central Europe [14].

According to these recommendations, there are preventive doses of vitamin D, that are taken on a daily basis throughout a year, but for summer months, with normal levels of 25(OH)D in the blood serum; marginal ones that will not lead to any side-effects when vitamin D medications are taken; treatment doses used in case of established vitamin D deficit and in case of different somatic diseases, including allergic diseases, since it is considered that hypovitaminosis D can be one of the factors contributing to the development of allergic pathology [15, 16, 17, 18, 19, 20].

Over the recent years statistical research on the spread of allergic diseases among children and adults has testified to their rise throughout the world. That urges to do further studies in order to substantiate and develop prophylactic and treatment measures for this category of patients. Research of the clinical and laboratory efficiency of applying vitamin D medications in different dosage in children with allergic diseases are promising as far as development of a long-term treatment scheme with inclusion of this medication to the basic therapy for this category of patients is concerned.

THE AIM

To study the dynamics of the level of 25(OH)D, IL-4, IL-10, and IgG in the blood serum of children with allergic diseases and to study the clinical effect of the application of vitamin D₃ in different dosage for this category of patients.

MATERIALS AND METHODS

The study has been done in compliance with the provisions of the Declaration of Helsinki of the World Medical Association on the ethical principles for medical research involving human subjects (as of 2008), with the consent of the biotic commission (protocol of the meeting No. 76 as of December 25, 2013). The research has been performed on the basis of the written consent of the parents or guardians of each examined child to the research.

We have examined 153 children with allergic diseases, aged 3-16. As of the date of examination the children did not have any acute respiratory infections and chronic infection sites that could influence the results of the laboratory studies. There were 69 patients with bronchial asthma 69 (46.9% ± 4.2%), 18 patients (11.2% ± 2.6%) had atopic dermatitis, 12 patients (7.0% ± 2.1%) had allergic rhinitis. There were 28 children with combined forms of allergic diseases (bronchial asthma and atopic dermatitis) (18.2% ± 3.2%), 26 patients (16.8% ± 3.1%) had both bronchial asthma and allergic rhinitis. Among all 123 clinical cases of bronchial asthma 11 cases (8.9% ± 2.8%) were the cases of persistent bronchial asthma of mild degree of severity, 89 (72.4% ± 4.3%) – persistent bronchial asthma of moderate degree of severity, and 23 (18.7% ± 2.3%) – of persistent bronchial asthma of grave degree of severity. Out of 46 clinical cases of atopic dermatitis – 12 (26.1% ± 3.8%) were of mild form, 25 (54.3% ± 2.5%) – of moderate severity, and 9 (19.6% ± 4.3%) – of grave form of disease under the SCORAD

index. Among 38 clinical cases of allergic rhinitis 26 cases (68.4% ± 3.6%) were the cases of round-the-year form, and 12 (31.6% ± 2.6%) – of the seasonal form.

Determination of the level of 25(OH)D in the blood serum was used as the indicator of sufficiency of vitamin D in the child organism. The level of 25(OH)D was determined using the electrochemiluminescence method at the Eleksys 2010 analyser (Roche Diagnostics, Germany) under the Cobas system test at the SI 'D. F. Chebotariov Institute of Gerontology of the National Academy of Medical Sciences of Ukraine'.

The level of IL-4, IL-10, and IgG was assessed using sets of reagents for enzyme-linked immunoassay in the immunology laboratory of SI 'Institute of Urology of the National Academy of Medical Sciences of Ukraine'.

During statistical analysis the median (mean) (Me) was used as the central trend measure, while lower and upper quartiles were used for dispersion (Q₁ and Q₃). To compare quantitative indicators between two groups of children with allergic diseases the Wilcoxon indicator (W) was used, through determination of the criterion of Wilcoxon signed ranks (Z), the difference was significant at $P < 0.05$; in case of multi-time contrasting of the selection of children with allergic diseases after their treatment with cholecalciferol in different doses the Friedman criterion was applied (χ^2), the difference was significant at $P < 0.05$. The relationship between the level of 25(OH)D in the blood serum and the level of allergic inflammation cytokines (IL-4, IL-10) in the blood of children with allergic diseases was studied using the correlation method, through determination of the Spearman's rank correlation coefficient (φ).

RESULTS

As the result of the data obtained, the average level of 25-hydroxycalciferol in children with allergic diseases made up 20.59 ng/ml (Me = 20.59; Q₁ = 16.63; Q₃ = 27.15)

After prescription of vitamin D₃ medication in the dose 2,000 IU daily over 2 months to children with allergic diseases, the mean level of 25(OH)D in the blood serum of patients after cholecalciferol intake made up 35.87 ng/ml (Me = 35.87; Q₁ = 25.73; Q₃ = 41.56).

With the beginning of the sunny season the intake of vitamin D₃ medications was terminated. After the summer period the mean rate of 25(OH)D in the blood serum was 35.13 ng/ml (Me = 35.13; Q₁ = 33.34; Q₃ = 43.88).

After the end of summer children with allergic diseases were prescribed higher doses of cholecalciferol – 4,000 IU daily during the disease remission and 5,000 IU daily during the acute period, with a 2-month course, combined with basic therapeutic measures applied over the period of remission and acute condition. After vitamin D₃ intake the mean level of 25-hydroxycalciferol made up 46.35 ng/ml (Me = 46.35; Q₁ = 43.45; Q₃ = 53.25).

In the contrasting of the initial level of 25(OH)D in the blood serum of patients after administration of 2,000 IU of vitamin D₃ over 2 months, after summer and after the intake of cholecalciferol in higher doses (4,000–5,000 IU) over 2

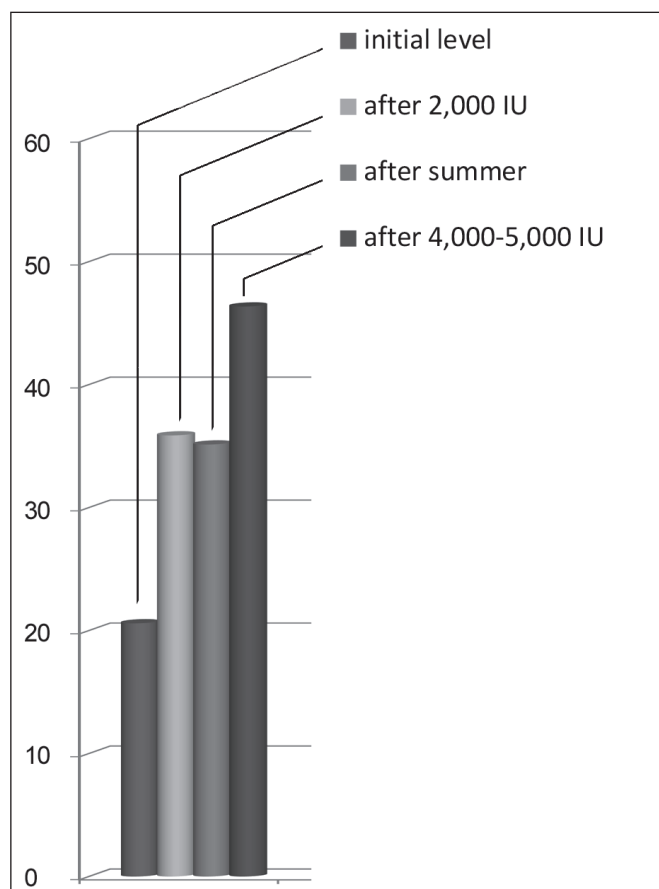


Fig. 1. Dynamics of the 25(OH)D level in the blood serum of children with allergic diseases depending on the administration of vitamin D₃ medication and the season of the year, a significant difference as contrasted with the initial level, $p < 0.05$

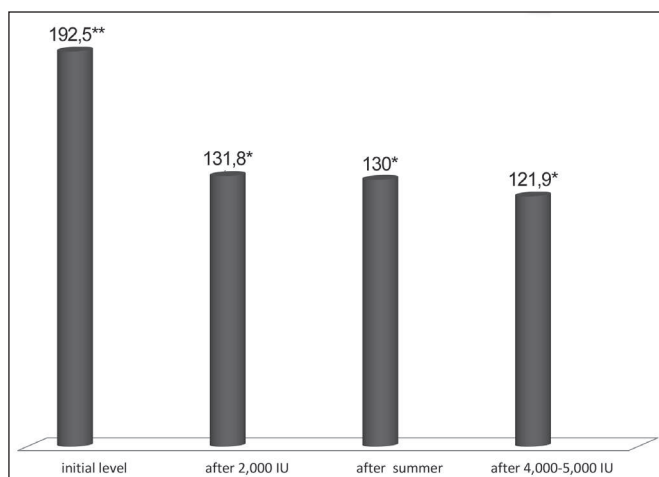


Fig. 2. Dynamics of IL-4 in children with allergic diseases in the acute period
Notes: * - significant difference as contrasted with the initial level, $p < 0.05$
** - significant difference as contrasted with the initial level, after 2,000 IU and after summer, $p < 0.05$.

months, a significant difference between the figures by the Friedman criterion was established ($\chi^2 = 41.211$; $P < 0.05$).

In the examination of the children under observation acute allergic disease was traced in 83 (54.3 ± 4.2 %), and remission – in 70 (45.7 ± 4.2 %) of children.

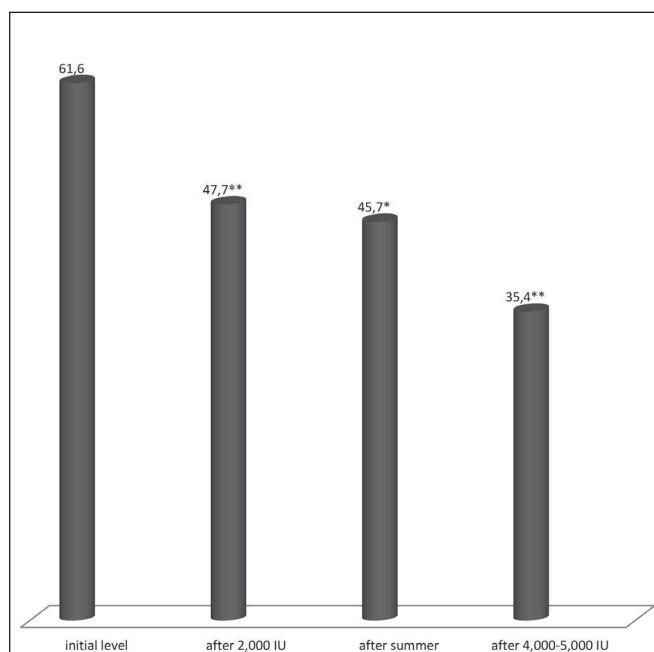


Fig. 3. Dynamics of IL-10 in children with allergic diseases in the acute period, * - significant difference as contrasted with the initial level, after 2,000 IU and after summer, $p < 0.05$

Notes: * - significant difference as contrasted with the initial level, $p < 0.05$
** - significant difference as contrasted with the initial level, after 2,000 IU and after summer, $p < 0.05$.

Below are the results of determination of immunological indicators in the blood of children with allergic diseases over the period of acute disease. Thus, initial level of IL-4 was 192.5 pg/ml ($Me = 192.5$; $Q_1 = 81.6$; $Q_3 = 257.6$), after cholecalciferol administration in the dose of 2,000 IU over 2 months – 131.8 pg/ml ($Me = 131.8$; $Q_1 = 82.1$; $Q_3 = 205.5$); after the summer period – 130.0 pg/ml ($Me = 130.0$; $Q_1 = 79.1$; $Q_3 = 175.6$), after vitamin D₃ medication administration in the dose of 4,000–5,000 IU over 2 months – 121.9 pg/ml ($Me = 121.9$; $Q_1 = 70.0$; $Q_3 = 143.9$). In the contrasting of IL-4 figures a significant difference was established between them ($P < 0.05$) (Fig. 2).

The initial level of IL-10 in children with allergic diseases in the acute period was 61.6 pg/ml ($Me = 61.6$; $Q_1 = 53.4$; $Q_3 = 77.9$), after intake of vitamin D₃ medication in the dose of 2,000 IU daily over 2 months – 47.7 pg/ml ($Me = 47.7$; $Q_1 = 40.4$; $Q_3 = 54.9$), after summer – 45.7 pg/ml ($Me = 45.7$; $Q_1 = 38.2$; $Q_3 = 53.8$), after cholecalciferol administration in the dose of 4,000–5,000 IU – 35.4 pg/ml ($Me = 35.4$; $Q_1 = 30.2$; $Q_3 = 44.5$). In the contrasting of IL-10 figures a significant difference was established between them ($P < 0.05$) (Fig. 3).

The initial IgG level over the acute period made up 28.1 g/l ($Me = 28.1$; $Q_1 = 15.1$; $Q_3 = 31.1$), after administration of vitamin D₃ medication in the dose of 2,000 IU over 2 months – 27.6 g/l ($Me = 27.6$; $Q_1 = 11.1$; $Q_3 = 32.2$), after summer – 21.7 g/l ($Me = 21.7$; $Q_1 = 14.7$; $Q_3 = 27.8$), after cholecalciferol administration in the dose of 4,000–5,000 IU over 2 months – 10.2 g/l ($Me = 10.2$; $Q_1 = 9.4$; $Q_3 = 14.4$). In the contrasting of IgG levels in children with allergic diseases a downward trend could be traced (Fig. 6).

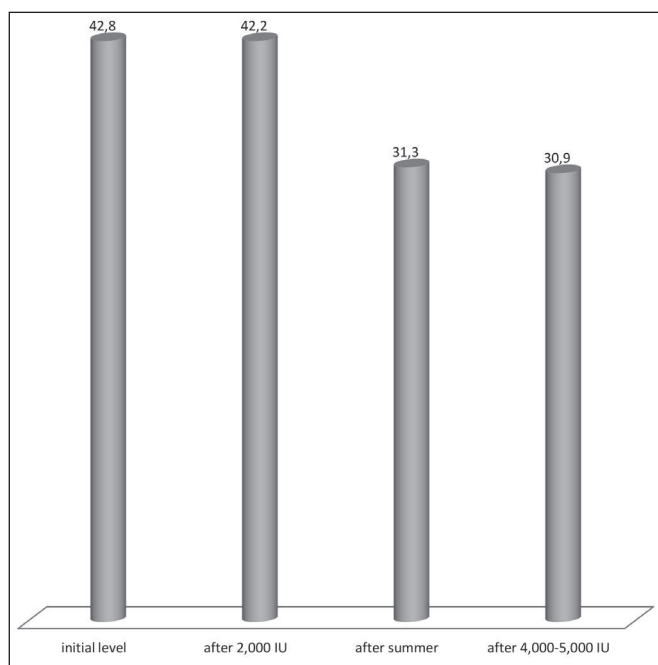


Fig. 4. Dynamics of the IL-4 level in children with allergic diseases in the remission period.

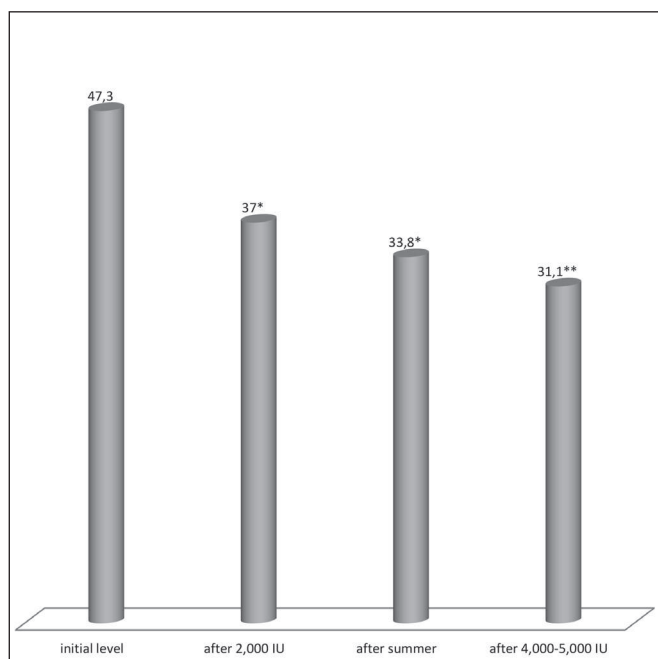


Fig. 5. Dynamics of IL-10 in children with allergic diseases in the period of disease remission

Notes: * - significant difference as contrasted with the initial level, $p < 0.05$
 ** - significant difference as contrasted with the initial level, after 2,000 IU and after summer, $p < 0.05$

Below are the results of determination of immunological indicators in the blood of children with allergic diseases in the period of disease remission. The initial level of IL-4 in the period of disease remission in children with allergic diseases made up 42.8 pg/ml (Me = 42.8; $Q_1 = 25.6$; $Q_3 = 111.2$), after cholecalciferol administration in the dose of 2,000 IU daily over 2 months – 42.2 pg/ml (Me = 42.2; $Q_1 = 28$; $Q_3 = 93$), after the summer period – 31.3 pg/ml (Me = 31.3;

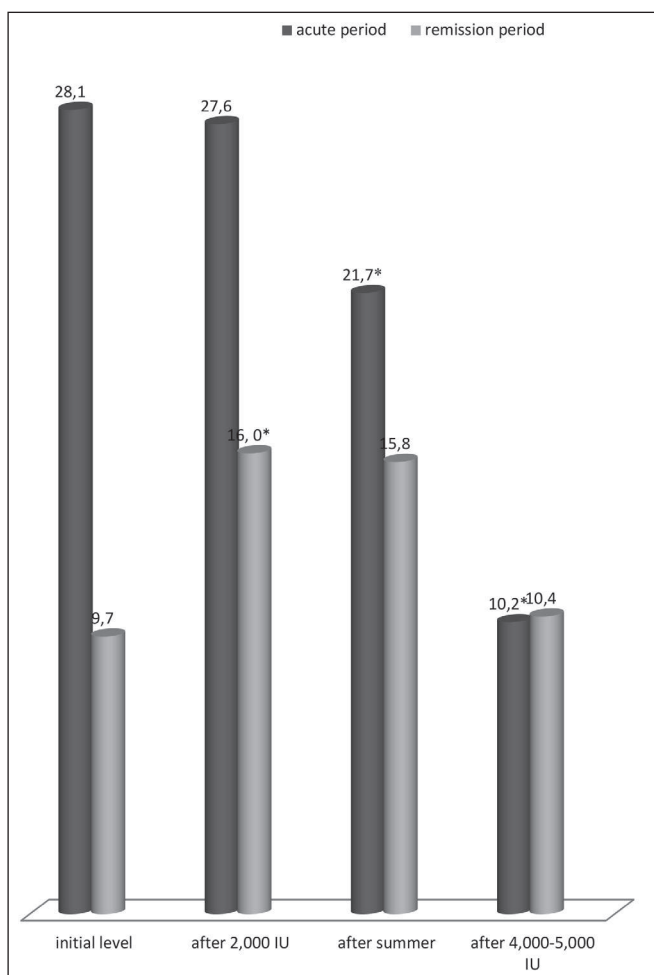


Fig. 6. Dynamics of IgG in children with allergic diseases in the acute period and in the period of disease remission.

Notes: * - significant difference as contrasted with the initial level, $p < 0.05$.

$Q_1 = 24.5$; $Q_3 = 86.3$), after intake of vitamin D₃ medication in the dose of 4,000–5,000 IU daily over 2 months – 30.9 pg/ml (Me = 30.9; $Q_1 = 20.1$; $Q_3 = 68$), a trend to decrease in the level of IL-4 in the blood of children in the period of disease remission could be traced (Fig. 4).

The initial level of IL-10 in children with allergic diseases made up 47.3 pg/ml (Me = 47.3; $Q_1 = 24.9$; $Q_3 = 62.2$), after administration of vitamin D₃ medication in the dose of 2,000 IU daily over 2 months – 37.0 pg/ml (Me = 37; $Q_1 = 24.4$; $Q_3 = 59.9$), after the summer period – 33.8 pg/ml (Me = 33.8; $Q_1 = 16.1$; $Q_3 = 54.7$), after administration of 4,000–5,000 IU of cholecalciferol a day within a 2-month course – 31.1 pg/ml (Me = 31.1; $Q_1 = 10.9$; $Q_3 = 50$). In the contrasting of IL-10 figures a significant difference between them was established ($P < 0.05$) (Fig. 5).

The initial level of IgG in the period of remission constituted 9.7 g/l (Me = 9.7; $Q_1 = 8.1$; $Q_3 = 20.1$); after administration of vitamin D₃ medication in the dose of 2,000 IU over 2 months – 16.0 g/l (Me = 16.0; $Q_1 = 9.7$; $Q_3 = 33.2$ g/l); after summer – 15.8 g/l (Me = 15.8; $Q_1 = 12$; $Q_3 = 31.4$); after administration of cholecalciferol in the dose of 4,000–5,000 IU over 2 months – 10.4 g/l (Me = 10.4; $Q_1 = 9$; $Q_3 = 12.5$). In the contrasting of IgG figures a significant difference between them was established ($P < 0.05$) (Fig. 6).

Table I. The course of allergic diseases before and after long-term administration of vitamin D₃ in the complex of treatment and rehabilitation activities in children

Feature	Prior to vitamin D ₃ administration	After vitamin D ₃ administration
Frequency of acute periods over the year	10–12 times	5–7 times
Frequency of acute periods over the year with hospitalization	5–10 times	3–7 times
ARI over the year	7–8 times	4–5 times
Duration of acute periods	10–14 days	8–6 days
Intoxication	Present	Almost non-available

In case of individual contrasting of the dynamics of the level of 25(OH)D in each child and the dynamics of IL-4, IL-10 in the blood against cholecalciferol arrival in the body from an endogenic and exogenic source in different periods of the disease, it was established that if the level of 25(OH)D in the blood serum goes up, the level of IL-4 and IL-10 goes down. Since for each child the fluctuations of IL-4, IL-10 and 25(OH)D were within the individual range, calculation of the correlation factor was made in accordance with each child's indicator, in particular, Spearman's rank correlation coefficient varied ($0.7 \leq \varphi \leq 1$), which fact testifies to a strong reverse correlation relationship.

In the course of the study, in order to maintain normal 25(OH)D level in the blood serum in children with allergic diseases after cholecalciferol prescription in higher doses, children with allergic diseases were recommended to continue intake of vitamin D₃ medication in the preventive dose – 1,000 IU daily over the year, but for summer months. All patients were kept under our observation over 1.5 years.

In the course of the study the therapeutic effect of administration of vitamin D₃ medication in children with allergic diseases could be traced, which fact is proven by the reduced number of acute allergic disease episodes and their severity over the year, reduced duration of acute condition, frequency of hospitalization due to the main disease over the year, reduced number of cases of acute respiratory infections over the year. In children with atopic dermatitis, when cholecalciferol was administered, reduced areas of skin lesion during the acute condition could be traced, as well as quick restoration of the skin cover in the period of convalescence, reduced number of secondary elements and skin dryness. Besides that, improved course of the main disease was also accompanied by improved general condition of patients, manifested in increased physical and mental activity, reduced manifestation of quick fatigue and weakness, reduced number of headache and dizziness episodes, improved appetite and sleep. The results of the clinical picture of patients are provided in table 1. It should be pointed out that a better manifested therapeutic effect could be traced when vitamin D₃ was administered in higher doses (4,000–5,000 IU) during 2 months in children with allergic diseases.

DISCUSSION

Taking into account due level of 25(OH)D in human blood serum and the results of the initial level of 25-hydroxycalciferol in children with allergic diseases, significant vitamin D

deficiency in the organisms of patients of this category has been traced. Thus, it may be considered that children with allergic diseases are prone to develop hypovitaminosis D.

In order to achieve normal level of 25(OH)D in the blood serum of patients, all children with allergic diseases regardless of their age were prescribed vitamin D₃ medication in the dose of 2,000 IU daily over 2 months. After that, on assessment of the organism provision with vitamin D in children with allergic diseases, normal level of 25(OH)D in the blood serum of patients was established. After two months of cholecalciferol intake the clinical effect in children with allergic diseases was difficult to assess due to short period of observation.

After the summer period the 25(OH)D level in the blood serum of children under examination remained almost the same. Taking into account the data on vitamin D metabolism, it may be assumed that vitamin D arriving to the body due to the medication intake before summer, over the summer period (3 months) should have left the organism, and the level of 25(OH)D in the blood serum of patients after summer should have gone back to its initial value. Obvious is the fact that synthesis of vitamin under the effect of solar UV-radiation can keep 25(OH)D in the blood serum of children at an adequate level after vitamin D₃ medication is administered. It should be indicated that no considerable improvement in the clinical course of allergic diseases could be traced.

In order to achieve the best possible level of 25(OH)D in the blood serum (36–48 ng/ml) of all patients and to raise the therapeutic effect for children with allergic diseases, higher doses of cholecalciferol were prescribed – 4,000 IU a day during remission of the disease and 5,000 IU a day during the period of acute disease in the course of 2 months, together with the basic treatment activities carried out in the periods of remission and acute disease. Higher doses of cholecalciferol were prescribed due to the recommendations on vitamin D medication administration in the residents of Central Europe. After that in no children the level of 25(OH)D in the blood serum was established as higher than the normal value (> 80 ng/ml), and no clinical signs of hypervitaminosis D were identified, also the best possible level of 25(OH)D in the blood serum of patients was achieved. Thus, it may be considered that prescription of higher doses of cholecalciferol within the above course for children with allergic diseases is safe.

Laboratory data related to provision of children with allergic diseases with vitamin D in the period of administration of its preparation in different doses shows that

cholecalciferol intake leads to increased level of 25(OH)D in the blood serum of patients, and the higher the vitamin D₃ medication dose, the higher the level of 25-hydroxicalciferol in the blood of patients.

To exclude the effect of the disease period on the level of immunological indicators in the blood of each patient they were measured over the same period of disease (remission or acute condition). To achieve this goal, children with allergic diseases were divided into two groups: children examined in the period of acute condition and children whose level of immunological indicators was measured in the period of remission.

It is known that IL-4 and IL-10 are cytokines of the immune system, that actively participate in the allergic inflammation processes, and then their increase in the blood of patients can be traced. Taking into account the results obtained, it may be assumed that vitamin D is involved in the decrease of allergic inflammation over the period of acute BA, allergic rhinitis and atopic dermatitis, since significant reduction in the level of IL-4, IL-10 and the trend to IgG decrease in the blood of children in the period of acute disease could be traced if vitamin D₃ was administered in different doses. If the IgG – IgG4 subpopulation is considered to be involved in the allergic inflammation, it may be assumed that the level of total IgG decreases as the result of this faction.

It may also be assumed that vitamin D reduces organism sensibilization in the period of remission of the allergic disease, which fact is confirmed by the trend to reduced level of IL-4, significant decrease of IL-10 and IgG content in the blood of children in the period of disease remission, if cholecalciferol is administered in different doses. In the contrasting of the indicators of the dynamics in the level of 25(OH)D and IL-4, IL-10 in the blood of every child a strong reverse correlation relationship was traced between the level of 25(OH)D and allergic inflammation cytokines (the higher the level of 25(OH)D, the lower the level of IL-4 and IL-10), which also confirms involvement of vitamin D in allergic inflammation decrease in different disease periods (acute condition, remission).

After the clinical effect was assessed in the period of cholecalciferol administration in different doses in children with allergic diseases, considerable improvement in the course of the disease was traced. This data proves the need for long-term administration of vitamin D₃ medication in this category of patients. The commencement of improved course of the main disease and the best possible level of 25(OH)D in the blood serum of patients could be traced after cholecalciferol prescription in higher doses (4,000-5,000 IU). It should be considered that short-term courses of treatment with vitamin D₃ in higher doses at the controlled level of 25(OH)D in the blood serum of patients should be included in the long-term cholecalciferol administration in children with allergic diseases. Reduced pathological symptoms that are not patogmonic for an allergic disease but may accompany it and characterize the overall condition of patients also point to the positive clinical effect of vitamin D. Also, reduced number of acute

respiratory disease episodes, which often trigger the development of acute allergic disease, over the year in children with allergic diseases should be pointed out, which fact also proves the need for cholecalciferol administration in this category of patients.

CONCLUSIONS

Thus, the results of the research show that vitamin D is involved in the immune system processes aimed at reduced allergic inflammation in the period of acute disease and disease remission, since significant reduction or a trend to reduced level of immunological indicators IL-4, IL-10, and IgG in the blood serum of children with allergic diseases in the period of disease remission and acute disease in case of their treatment with vitamin D₃ have been traced. The reverse correlation has been established between the level of 25(OH)D and IL-4, IL-10 in the blood serum of patients. Taking into account the data obtained, we consider that while in the treatment of children with bronchial asthma, allergic rhinitis and atopic dermatitis the complex therapy should include vitamin D₃ medications with a long course of treatment in different doses, including higher doses, at the controlled level of 25(OH)D in the blood serum of patients. That will contribute to improved course of the main disease and reduced manifestations of asthenoneurotic syndrome. The results of the research testify to the need for further scientific research into possible administration of vitamin D₃ in the treatment and prevention of allergic diseases in children.

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ORIGINAL ARTICLE

CLINICAL USEFULNESS OF THE INTERNATIONAL RENAL RESEARCH INSTITUTE OF VICENZA (IRRIV) SCORE IN THE INTENSIVE CARE SUBJECTS WITH RENAL FAILURE: SINGLE-CENTRE EXPERIENCE

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The aim: Acute kidney injury (AKI) is a common and clinically important condition that affects both kidney structure and function. International Renal Research Institute of Vicenza (IRRIV) score has been designed to enable early identification of patients who may require renal replacement therapy (RRT). We aimed to assess the usefulness of the IRRIV score in predicting the outcome in the intensive care unit (ICU) patients who may require renal replacement therapy (RRT).

Material and Methods: This retrospective study screened 955 consecutive patients hospitalized in a mixed tertiary ICU between Jan 2015 and Jul 2018. Patients with sCr > 3.5 mg/dl on the first 24 hours post-admission constituted the study group 1 (G1, n=54). Subjects who underwent RRT based on indications other than elevated sCr level were a study group 2 (G2, n=31). ICU mortality, a need for RRT and ICU length of stay (LoS) were the outcomes.

Results: Median IRRIV score was 5.5 points (IQR 4.5-6.5) in G1 and 3.5 points (IQR 3-5.5) in G2. IRRIV score poorly predicted the need for RRT implementation (AUC=0.652, 95%CI 0.510-0.776, P=0.048). The IRRIV score failed to predict mortality in both groups (G1: AUC=0.610, 95%CI 0.468-0.740, P=0.16; G2: AUC=0.530, 95%CI 0.343-0.710, P=0.79). No correlation was found between the score and ICU LoS (G1: R= -0.13, P=0.36; G2: R= -0.27, P=0.15).

Conclusions: The retrospective analysis of our regional data did not confirm the expected usefulness of the IRRIV score in predicting the need for RRT nor in the prognostication of the patients admitted to the ICU due to renal failure.

KEY WORDS: acute kidney injury, International Renal Research Institute in Vicenza score, renal replacement therapy

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INTRODUCTION

Acute kidney injury (AKI) constitutes a serious clinical condition that affects both kidney structure and function. It may concern 30-40% of patients treated in the intensive care units (ICU) worldwide [1, 2]. There are a plethora of AKI risk factors [3, 4]. AKI may significantly affect prognosis [5, 6].

According to AKIN (Acute Kidney Injury Network) [7] or KDIGO (Kidney Disease: Improving Global Outcomes) [3] guidelines, AKI is classified into stages which are in direct correlation with progression of renal insufficiency [3]. The optimal timing of implementation of renal replacement therapy (RRT) for AKI in ICU subjects is still not well defined and the decision must be made based on clinical and biochemical parameters of volume overload, azotaemia, hyperkalaemia, and acidosis [1, 2, 8]. These factors are considered "classic" indications for RRT [9]. Although AKIN 3 stage or AKIN 2 stage with a documented renal damage (by biomarkers) are believed to be the best thresholds for RRT to start [10, 11], there is some evidence that even earlier treatment may improve the outcome in critically ill [12-14]. However, in contrast to the studies promoting the earlier start of RRT, examples of recent trials point towards a favorable strategy of delayed initiation or cannot give an unequivocal answer [15, 16].

Several attempts have been made to establish a simple but accurate method of facilitating decision making. IRRIV (International Renal Research Institute of Vicenza) score has been designed to enable early identification of vulnerable patients who may require RRT [17]. Although the first results seemed promising, its clinical utility has not been yet validated externally.

THE AIM

We aimed (1) to assess the usefulness of the IRRIV score in the prediction of RRT implementation in patients with elevated sCr concentration, (2) to verify its utility in patients presenting "classic" indications for RRT, and (3) to verify whether the score helps predict the outcome in the ICU setting.

MATERIAL AND METHODS**STUDY POPULATION**

In this retrospective study, a number of 955 consecutive patients hospitalized between 01.2015 and 07.2018 in a mixed ICU was screened. Then, 865 individuals were excluded due to sCr level

Table I. Study group characteristics and procedure-related variables.

Variable	Category/Unit	G1 (n=54)	G2 (n=31)	Combined G1+G2 (n=85)
Sex	Males	31 (57%)	13 (42%)	44 (52%)
	Females	23 (43%)	18 (58%)	41 (48%)
Age	(years)	61 (48-72)	65 (52-71)	64 (50-72)
APACHE II	(points)	21 (16-29)	21.5 (15-28)	21 (15-29)
Serum creatinine on day 1 of ICU admission	(mg/dl)	3.5 (2.08-4.42)	1.63 (1.17-1.95)	2.42 (1.47-3.70)
eGFR on day 1 of ICU admission	(ml/min/1.73m ²)	18 (12.7-32.3)	44.7 (29.6-56.6)	23.8 (16.2-48.1)
Length of ICU stay	(days)	6.5 (3-18)	8 (3-19)	7 (3-19)
Length of RRT	(days)	3 (2-4)	5 (2-6.3)	4 (2-6)
Chronic Kidney Disease (CKD) stage	Median (IQR)	4 (4-5)	0	4 (4-5)
	G1	0	0	0
	G2	0	0	0
	G3a	2 (3.7%)	0	2 (2.4%)
	G3b	1 (1.9%)	0	1 (1.2%)
	G4	6 (11.1%)	0	6 (7.1%)
	G5	8 (14.8%)	0	8 (9.4%)

Values are medians, interquartile ranges (Q1–Q3) for quantitative variables, and frequencies and percentages for qualitative variables

Table II. Causes of acute kidney injury in both groups (according to KDIGO [3]).

Risk factors/ Exposures	G1 N=54	G2 N=31	Risk factors/ Susceptibilities	G1 N=54	G2 N=31
Sepsis	7 (13%)	8 (25.8%)	Dehydration/ volume depletion	12 (22.2%)	7 (22.6%)
Critical illness	54 (100%)	31 (100%)	Advanced age (> 65yrs)	23 (42.6%)	14 (45.2%)
Circulatory shock	9 (16.7%)	4 (12.9%)	Female gender	23 (42.6%)	18 (58.1%)
Trauma	5 (9.3%)	0	Chronic kidney disease	17 (31.5%)	0
Major non-cardiac surgery	16 (29.6%)	8 (25.8%)	Chronic disease (heart, lung, liver)	24 (44.4%)	20 (64.5%)
Nephrotoxic drugs	1 (1.9%)	1 (3.2%)	Diabetes mellitus	12 (22.2%)	8 (25.8%)
Radiocontrast agents	4 (7.4%)	6 (19.4%)	Cancer	16 (29.6%)	8 (25.8%)
Poisonous plants and animals	0	0	Anaemia (F: <12 g/dl; M: <13 g/dl)	29 (53.7%)	19 (61.3%)

Values are frequencies and percentages for qualitative variables

<3.5 mg/dl and/or no other existing indications for RRT. From the group of remaining 90 subjects, five more patients were excluded from further evaluation (length of ICU stay shorter than 24h, n=2; transfer to another hospital/unit, n=2; lack of data, n=1). Patients' flowchart is depicted in Figure 1.

Patients with sCr $\geq$ 3.5 mg/dl on the first 24 hours post-ICU-admission (as an accepted indication for RRT implementation [18]) constituted the study group "1" (G1, n=54). Subjects who underwent RRT based on occurring indications, regardless of the recorded sCr level staying within reference values, were considered the study group "2" (G2, n=31). The rationale for RRT start in G2 was as follows: acidosis (n=28), anuria (n=18), fluid overload (n=11), sepsis (n=8), and toxic acute renal failure (n=5). Groups G1 and G2 were not interchangeable, meaning no patients from G2 had

sCr>3.5 mg/dl during the entire length of ICU stay. 17 patients in G1 and 0 patients in G2 had chronic kidney disease (CKD) diagnosed before the ICU admission. However, none of the patients required RRT prior to the ICU admission analyzed in our study. All subjects had continuous RRT (CRRT), either continuous veno-venous hemodialysis (CVVHD, n=27), continuous veno-venous hemodiafiltration (CVVHDF, n=20) or continuous veno-venous hemofiltration (CVVHF, n=1).

DATA EXTRACTION AND ANALYSIS

Medical and demographic data were retrieved from medical records, with special attention given to the acknowledged AKI risk factors, according to KDIGO guidelines [3], and the acknowledged indications for RRT [9], i.e. excessive fluid over-

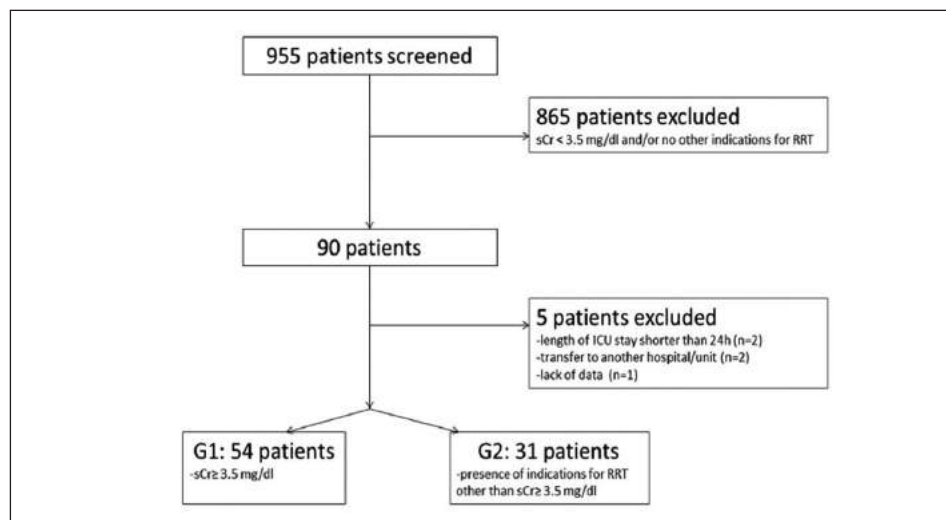


Fig. 1. Flowchart of the study population.

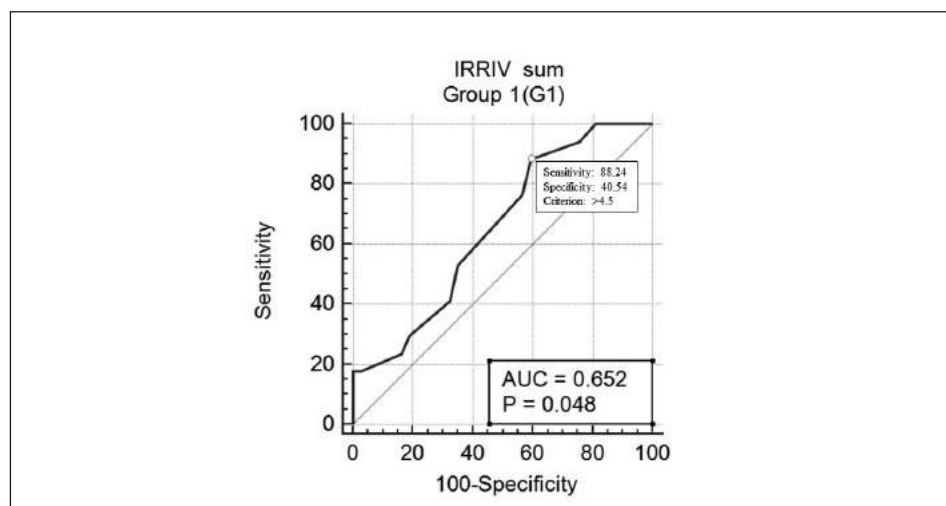


Fig. 2. Prediction of RRT need using the IRRIV score in G1 patients.

Table III. Parameters of the IRRIV score in both groups (according to Zaragoza et al. [15]).

Parameter	G1 N=54	G2 N=31
Mean arterial pressure (lowest) ≤ 65mmHg	26 (48.1%)	19 (61.3%)
Body temperature (highest) ≥ 38.2°C	5 (9.3%)	3 (9.7%)
HCO ₃ ⁻ concentration (lowest) ≤ 23 mmol/l	39 (72.2%)	23 (74.2%)
Urinary output (lowest) ≤ 40 ml/h	30 (55.6%)	23 (74.2%)
SOFA renal (highest) ≥ 2	42 (77.8%)	8 (25.8%)
No mechanical ventilation	9 (16.7%)	3 (9.7%)
Change of sCr ≥ 0.3 mg/dl	49 (90.7%)	18 (58.1%)
Fluid accumulation ≥ 10%	15 (27.8%)	11 (35.5%)

Values are frequencies and percentages for qualitative variables. SOFA- Sequential Organ Failure Assessment score, sCr- serum creatinine.

load (>10%), acidosis (pH<7.15), low urine output (< 0.5 ml/kg/h for at least 6 hours), sepsis, and acute renal failure (AKIN class 3). IRRIV score for each patient was calculated based on

the worst recorded value during the first 24 hours after patient's admission to the ICU with some complementary data taken from the patient's further ICU stay (e.g. sCr value from the day of ICU discharge). The score consists of 8 parameters with a threshold value assigned for each of them, i.e. mean arterial pressure, body temperature, serum HCO₃⁻ concentration, urinary output, Sequential Organ Failure Assessment (SOFA) renal score, need of mechanical ventilation, change of serum creatinine (sCr) during hospital stay, and fluid accumulation [17]. During the evaluation, a sum of a maximum 11 points could be given to the patients based on their clinical status and laboratory results. Each parameter has a one ascribed value of 1, 1.5 or 2 points exactly. The threshold of >3.5 points (including at least one of the renal dysfunction markers) has been accepted as an indication for RRT. [18]

ICU mortality and ICU length of stay (LoS) were considered the outcome. ICU LoS was defined as the time from the ICU admission to either the patient's discharge from the hospital, transfer to another ward or death.

ETHICAL ISSUES

Under section 21 and 22 of the Act of 5 December 1996 on the Medical Profession [19], due to non-interventional and

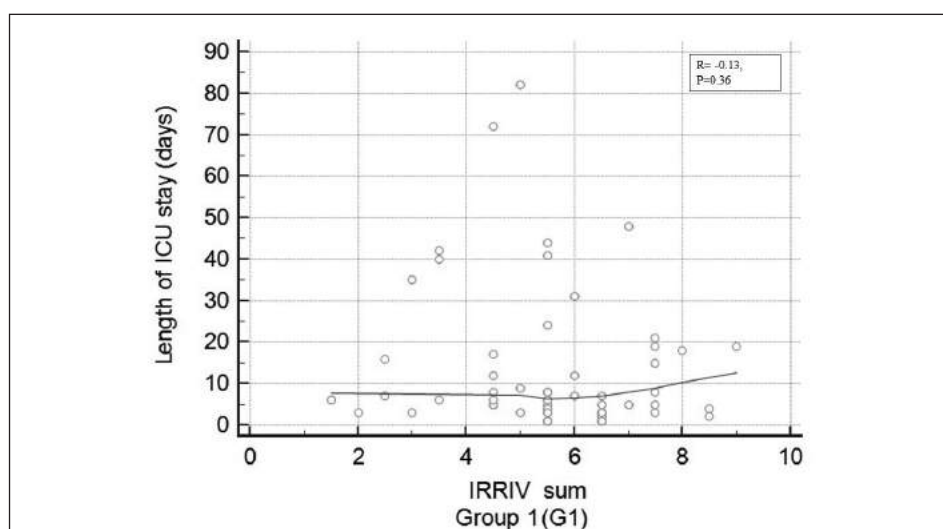


Fig. 3. Scatter diagram for the association between the IRRIV score and ICU length of stay in G1 patients.

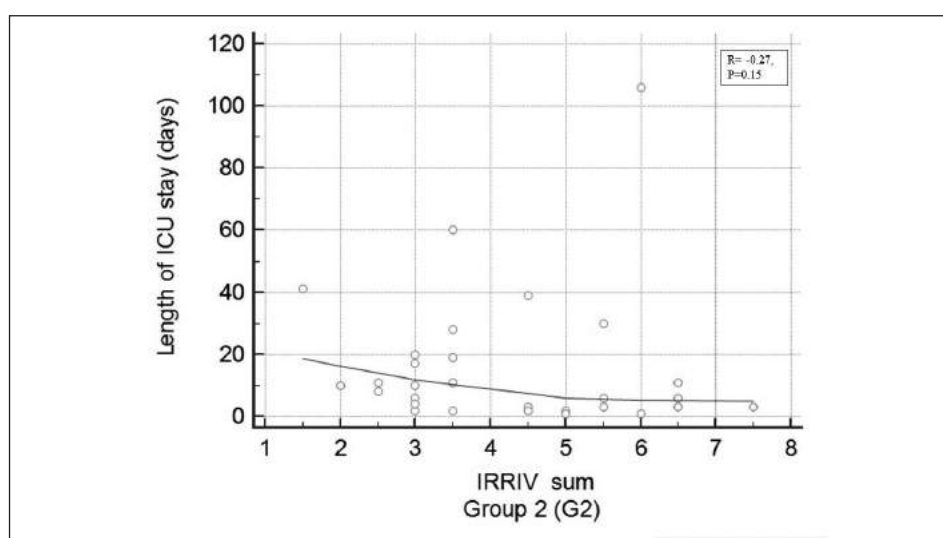


Fig. 4. Scatter diagram for the association between the IRRIV score and ICU length of stay in G2 patients.

retrospective design of the study, no approval of the Ethics Committee was required. However, all patients data were obtained in accordance with the national law regulations of personal data management, after the written consent was given by the patients on hospital admission, excluding unconscious patients who required emergency procedures.

STATISTICAL ANALYSIS

Statistical analysis was performed using MedCalc v.18 software (MedCalc Software, Ostend, Belgium). Quantitative variables were depicted using medians and interquartile ranges (IQR, i.e., 25pc–75pc). The D'Agostino-Pearson test was used to verify their distribution. Qualitative variables were described with frequencies and percentages. Between-group differences for continuous variables were assessed using the Kruskal-Wallis test, while for categorical variables the Chi-squared test was applied. ROC curves were drawn and areas under the ROC curves (AUC) were calculated to determine the predictive value of the IRRIV score and the outcome. Correlations were assessed using Spearman's rank coefficient. A p-value <0.05 was considered significant.

RESULTS

G1 consisted of 54 patients (31 men, 57% / median age of 61 years, IQR 48-72). G2 included 31 patients (13 men, 42% / median age of 65 years, IQR 52-71). Median baseline APACHE II score was 21 (IQR 16-29) in G1 and 21.5 (IQR 15-28) in G2, respectively (P=0.89).

Detailed characteristics of the study groups are depicted in Table I. Distribution of classic AKI risk factors in both groups is depicted in Table II. Median IRRIV score was 5.5 points (IQR 4.5-6.5) in G1 and 3.5 points (IQR 3-5.5) in G2 (P=0.02). Distribution of all constituents of the score in both groups is shown in Table III.

In G1, 17 patients (32%) had CRRT during ICU stay. Median RRT time was 3 days (IQR 2-4.5) in G1 and 5 days (IQR 2-6) in G2. Median ICU LoS was 6.5 days (IQR 3-18) in G1 and 8 days (IQR 3-19) in G2, respectively (P=0.54). Mortality was higher in G2 (n=20, 64.5%) compared to G1 (n=20, 37%) (P=0.015).

IRRIV score poorly predicted the need for RRT implementation in G1 (AUC=0.652, 95%CI 0.510-0.776, P=0.048) (Fig. 2). No correlation was found between IRRIV score and ICU LoS (G1: R= -0.13, P=0.36; G2: R= -0.27, P=0.15).

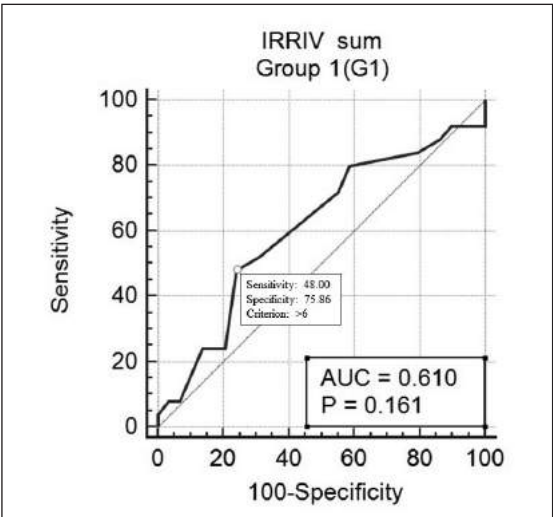


Fig. 5. Prediction of mortality using the IRRIV score in G1 patients.

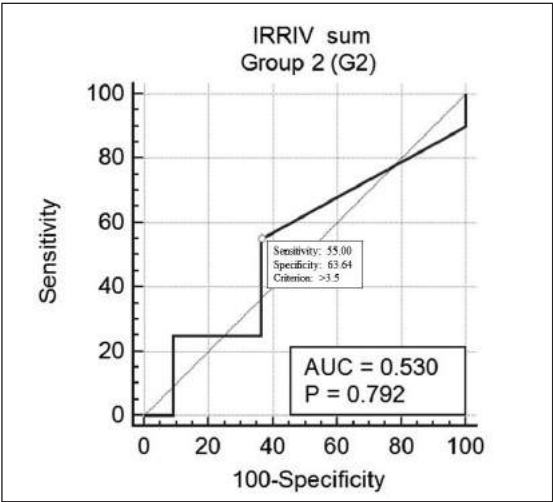


Fig. 6. Prediction of mortality using the IRRIV score in G2 patients.

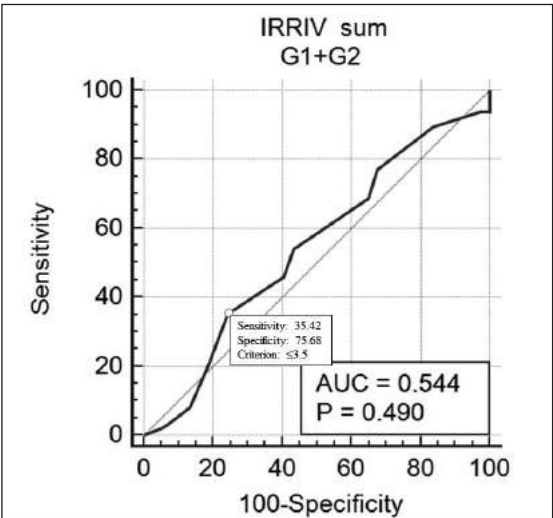


Fig. 7. Prediction of RRT need using the IRRIV score in combined G1 and G2 patients.

(Fig. 3-4). IRRIV score failed to predict mortality in both groups (G1: AUC=0.610, 95%CI 0.468-0.740, P=0.16; G2: AUC=0.530, 95%CI 0.343-0.710, P=0.79) (Fig. 5-6).

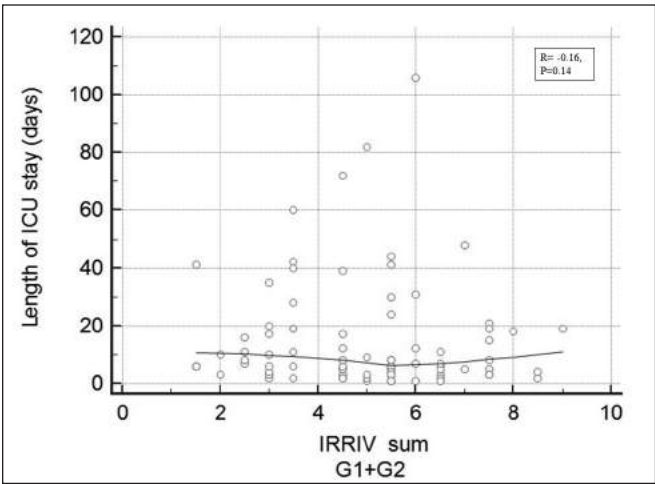


Fig. 8. Scatter diagram for the association between the IRRIV score and ICU length of stay in combined G1 and G2 patients.

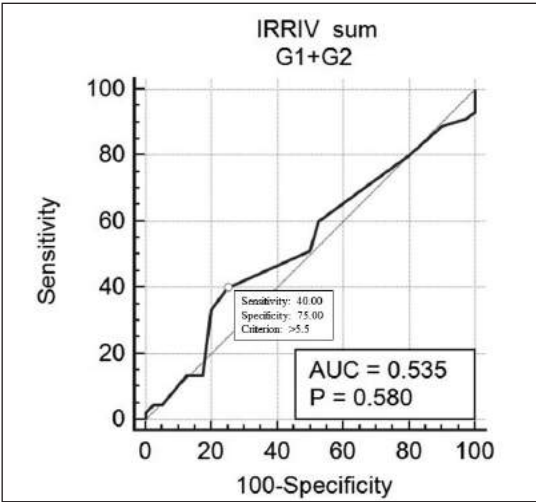


Fig. 9. Prediction of mortality using the IRRIV score in combined G1 and G2 patients.

When combined G1 and G2 were analyzed, IRRIV could not predict the need for RRT implementation (AUC=0.544, 95%CI 0.433-0.653, P=0.490) (Figure 7). No correlation was found between IRRIV score and ICU LoS (R= -0.16, P=0.14) (Figure 8). IRRIV score failed to predict mortality (AUC=0.535, 95%CI 0.424-0.644, P=0.58) (Figure 9).

DISCUSSION

In this short study, we sought to verify the clinical utility of the IRRIV score in the local academic ICU. The score predicted neither a need for RRT implementation nor the short-term outcome.

Our project was inspired by the original study of Zaragoza et al. who analysed the frequency of RRT, ICU mortality and length of ICU stay [17]. Interestingly, our results are not in line with those from a Vicenza group. In their paper, the score helped predict a need for RRT (AUC=0.81), mortality (P<0.001; AUC not calculated), and LoS (P<0.001). There are, however, some discrepancies between the studies in terms of the number of subjects, mode of participants'

selection, and distribution of baseline AKI risk factors (sCr, SOFA renal score) which make direct comparisons impossible. The original study population comes from the NEFROINT (NEFRologia e Cura INTensiva) Investigation group database which is a prospectively collected database of 10 Italian ICUs and it was focused on the epidemiology and risk factors of AKI in the ICU [20,17]. Our patients came from a single-centre, mixed ICU, therefore our study group included patients in a critical state, with disorders of various backgrounds e.g. medical, post-operative, neurological, etc. one ought to remember that we didn't aim to monitor the exact number of parameters as recorded by the Vicenza team, but we directly based on a final model constructed by them. Our investigation generally focused less on the time and events prior to the ICU hospitalization, e.g. pre-hospitalization sCr levels and previous exposure to toxics, which usually are difficult to assess. Moreover, Zaragoza and colleagues implemented a control group of patients with a relatively low serum creatinine level (mean value of 0.9 mg/dl). The rationale to investigate the G1 group in our study was to check if the score was truly decisive in starting RRT in "borderline" patients with elevated creatinine concentration. As a result, we chose the critically ill patients whose sCr was high enough to suggest the need for renal support. Then we evaluated the subsequent IRRIV score parameters to confirm the need for therapy and checked if it was indeed delivered.

Unfortunately, there is a paucity of international data enabling further comparisons regarding the utility of the IRRIV score. Particularly, there is a lack of evidence on validation of the score in different ICU populations (i.e. surgical, septic, cardiac) or in different clinical scenarios (i.e. non-ICU). Our paper is not an exact copy of the original study, rather a reimagination aimed at congruent goals. From this point of view, we believe our study to be novel in this field.

Despite the fact that RRT has become an integral part of modern critical care, identifying the optimal timing and optimal indications for RRT remain difficult in ICU practice. Unfortunately, the findings of recently published meta-analyses are inconclusive. Including 9 RCTs, Lai et al. [21] revealed that compared with "later" RRT, "earlier" initiation of treatment did not show beneficial impacts on patient outcomes. However, a lower rate of death was observed among surgical patients and in those who underwent CRRT. No beneficial effect of "earlier" start of RRT was found by Yang and colleagues in their comprehensive meta-analysis of RCTs [22]. Besen et al. [23] revealed that early initiation of RRT was associated with lower mortality in prospective and retrospective observational studies, however, after excluding low-quality papers, the relationship remained insignificant. Also, a meta-analysis with trial sequential analysis of RCTs documented no benefit of the early use of RRT [24]. There is a serious methodological problem of definition of "early" and "late" RRT implementation, which differs significantly between studies. We may, therefore, assume that conclusions on the relationship between RRT timing and the outcome are

biased, and the additional value of the IRRIV score is of low importance in this context.

STUDY LIMITATIONS

One should bear in mind potential limitations of our study, including relatively small study group with high sCr, lack of a control group with patients only "at risk" of RRT (AKIN stage 1 and 2), lack of calculated AUC values for each independent IRRIV score parameters due to limited number of subjects, no precise information regarding the entire population of 955 ICU patients with regard to AKI risk factors, limited number of variables included in statistical analysis (i.e. cause of admission), resulting in unclear determination as to what extent they affected the outcome in logistic regression. It also needs to be underlined that a relatively low percentage of subjects received RRT. Nonetheless, out of 955 examined cases, only the chosen 85 presented any indications for renal support.

CONCLUSIONS

We may conclude that the retrospective analysis of our regional data did not confirm the expected usefulness of the IRRIV score in predicting the need for RRT nor in the prognostication of the patients admitted to the ICU due to renal failure.

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Conflicts of interest:

Authors declare no conflicts of interest.

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D – Writing the article, E – Critical review, F – Final approval of the article

ORIGINAL ARTICLE

ASSESSMENT OF THE ADAPTIVE PERSONAL POTENTIAL OF THE PATIENTS WITH PARANOID SCHIZOPHRENIA

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ABSTRACT**The aim:** assessment of the patients with paranoid schizophrenia adaptive personal potential.**Materials and methods:** Clinical-anamnestic method, questionnaires for determining "structural ego-states" by J. Hey, of the "drivers" by M. Cox, of the "personal adaptations" by Joines, for determining the "crisis moments of development" by J.I. Clark, statistical analysis of the results data.

164 patients with a newly diagnosed and episodic paranoid schizophrenia took part in this study.

Results: "Passive behavior" serves as a typical door for contact by 88% of the patients. According to the methodologies of determination of the "drivers" the following results were received: "make the others happy" – 28%, "be strong" – 76%, "be perfect" – 65%, "make efforts" – 5% "hurry up" – 15%; of "crisis moments in development" determination, 61% were determined as "stuck" in the 1st stage of the development. By 74%, there was a "script prohibition", "do not be close." By 72% there was a "prohibition" "do not have feelings". The prevailing combinations of the "personal adaptations" were "schizoid" (92%) and "paranoid" (81%).**Conclusions:** schizophrenia can be viewed as a defensive reaction. The "ego- states of the controlling parent" and "adaptive child" dominate. The dominant "drivers" are "make the others happy" and "be strong." Typical "script prohibitions" are "do not be close," "do not feel," "do not think" and "do not be healthy." The typical "personal adaptations" of the patients are "schizoid" and "paranoid".**KEY WORDS:** personality structure, transactional analysis, psychosocial rehabilitation

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INTRODUCTION

There are important changes in the world psychiatry associated with understanding and awareness of the significant role of rehabilitation in the complex treatment of the mental illnesses of the schizophrenic spectrum. The most important for such patients are increase in neurocognitive deficit and complex psychological problems in the family, associated with the risk of its breakdown, stigmatization by the society with the social "displacement" of the patient and overall decline in the quality of his/her life [1].

The practicing psychiatrists have the necessity of awareness the importance of including a complex combination of psychopharmacotherapy, psychotherapeutic and social rehabilitation methods approaches in the treatment program of schizophrenia at all stages of the therapy. These approaches were included in the clinical protocols for providing medical care to the patients with schizophrenia [2,3,4,5]. The transactional analysis (TA) found its place in the system of rehabilitation measures [6, 7].

The pathodynamic component of the adaptive personal potential of the patients with schizophrenia includes such characteristics as the rate of progression, the relevance of psychopathological rueful feelings, the ratio of the positive and deficient symptoms, the phenomena of disorganization of the mental processes and mechanisms for compensating disorders at the clinical-psycho-pathological level [8].

The problem of psychotherapy of the patients with schizophrenia is relevant to the modern society, which seeks to socialize and ensure the adequate standard of living for everyone. The transactional analysis proved to be an effective method for psychotherapy of such patients, but it lacks theoretically grounded and statistically confirmed data on the characteristics of the structure and personality development, characteristic behavioral patterns, components of the internal picture of the disease by schizophrenia, necessary to improve the system of the effective psychotherapy. From the perspective of the transactional analysis, the patient is the subject of psychotherapy in the sequential development of the structure of his/her personality structure. Of course, such an integrative approach is a definite novelty compared with the discrete-descriptive diagnostic approach, which due to well-known historical and social circumstances is ingrained in the domestic psychiatry and clinical practice.

THE AIM

Assessment of the adaptive personal potential of the patients with paranoid schizophrenia. The object of this study was to determine the structure of the personality as a component of the adaptive personal potential of the patients with the paranoid form of schizophrenia. The subject of the study were the factors of forming the internal picture

of the disease by the patients with the paranoid form of schizophrenia. The task of the study was to investigate the factors that form the internal picture of the disease in the paranoid form of schizophrenia using psychodiagnostic methods to determine the characteristics of the personality structure.

MATERIALS AND METHODS

Theoretical analysis of the current state of the problem under study, clinical and anamnestic method, questionnaire for determining “structural ego-states” by Julia Hey, questionnaire of the “drivers” by Mary Cox, questionnaire of the “personal adaptations” by Joines, questionnaire for determining the “crisis moments of development” by Jean Isily Clark, statistical analysis of the results data. In the course of the study, the patients with CG (control group) No.2 and SG (study group) No.2 received psychotherapeutic correction in the form of group psychotherapy using the transactional analysis three times a week. According to the conclusions of the examination of the ethics committee of the university during the course of this study, all the main provisions of biomedical ethics were observed.

RESULTS

64 patients with a first-time diagnosis of paranoid schizophrenia and paranoid schizophrenia with episodic course took part in this study, in which 91 – women and 73 – men, the average age of the patients made up $29,25 \pm 13,5$ years, the total number of the patients with the diagnosis “paranoid schizophrenia” – 118, with the diagnosis “acute polymorphic psychotic disorder with symptoms of schizophrenia” – 46 patients, which by re-hospitalization, had the diagnosis of “paranoid schizophrenia”, the average illness duration – $2,7 \pm 2,1$ years.

There were 4 groups of patients formed, depending on the received anti-psychotic and psychotherapy: the study group No.1 (SG) included the patients who received anti-psychotic therapy with atypical anti-psychotics with pronounced sedative action (quetiapine), with activating action (aripiprazol, risperidone, amisulpride, sertindol); the study group No.2 (SG + PC) (psychotherapeutic correction) included the patients who received anti-psychotic therapy with typical anti-psychotics with pronounced sedative action (quetiapine) and with activating action (aripiprazol, risperidone, amisulpride, sertindol) and psychotherapeutic correction in the form of group therapy using the transactional analysis; the control group No.1 (CG) included the patients who received anti-psychotic therapy with atypical anti-psychotics with pronounced sedative action (chlorpromazine, levomepromazine, flufenazine, flupentixol, zuclopentixol) and with activating action (butyrophenone, trifluoperazine, thioridazine); control group No.2 (CG + PC) included patients receiving anti-psychotic treatment by the typical anti-psychotics with pronounced sedative action (chlorpromazine, levomepromazine, flufenazine, flupentixol, zuclopentixol) and with activating action

(butyrophenone, trifluoperazine, thioridazine) and psychotherapeutic correction in the form of group therapy using the transactional analysis.

In the course of the examination, the following dominant functional “ego-states” were identified by the patients under study Fig. 1 15 (60%) patients of CG + PC and 28 (68%) patients of SG + PC had “ego-state of the controlling parent”; 0% of the patients of CG + PC and 0% of the patients of SG + PC – “ego-state of the caring parent”; 5 (12%) patients of CG + PC and 5 (12%) patients of SG + PC – “ego-state of the adult”; 5 (20%) patients of CG + PC and 8 (20%) patients of SG + PC – “ego-state of the adaptive child”; 0% of the patients of CG + PC and 0% of the patients of SG + PC – “ego-state of the free child”.

The typical doors “for contact”, by Paul Ware, were assigned as “passive behavior” by 22 (88%) patients of CG + PC and 36 (87%) patients of SG + PC.

3 patients (12%) of CG + PC and 5 (13%) patients of SG + PC make contact with the outside world through its understanding.

According to the test methods of determination of the leading “drivers” by Mary Cox by the persons under study the following combinations of the behavioral partners or “drivers” were determined Fig. 2: “make others happy” by 7 patients (27%) of CG + PC and 12 (28%) of SG + PC, “be strong” by 18 (72%) of CG + PC and 32 (76%) of SG + PC, “be perfect” by 15 (60%) of CG + PC and 27 (65%) of SG + PC, “make efforts” by 2 (6%) of CG + PC and 2 (5%) of SG + PC and “hurry up” by 3 (12%) of CG + PC and 6 (15%) of SG + PC.

During the conducted methods for assigning the “crisis moments of the development” by Jean Isily Clark by 17 (67%) of the persons of CG + PC and by 25 (61%) of SG + PC under study there was “stuck” on the 1st stage of development. By 18 (71%) patients of CG + PC, and by 30 (74%) of SG + PC, there was a “script prohibition” “do not be close” found.

By 17 (67%) of the examined patients of CG + PC and by 29 (72%) of SG + PC the “prohibition” “do not have your feelings” was revealed. The “prohibition” “do not think” revealed by 14 (55%) patients of CG + PC and also by 20 patients (48%) of SG + PC. By 14 (55%) patients of CG + PC and by 25 (61%) of SG + PC, the “prohibition” “do not be healthy” was revealed that they materialized getting sick with schizophrenia.

According to the criteria for determination of the “personalized adaptations” the most prevalent combinations of patients’ “personalized adaptations” were determined: “schizoid” – by 21 (85%) of CG + PC and by 38 (92%) of SG + PC and “paranoid” – by 19 (76%) of CG + PC and by 33 (81%) of SG + PC.

DISCUSSION

The negative manifestations of attention, which were called “strokes” by Berne, are the reflection of the negative “parent ego-state” of the parenteral figures and therefore form the negative parent introject by the persons under study. Thus,

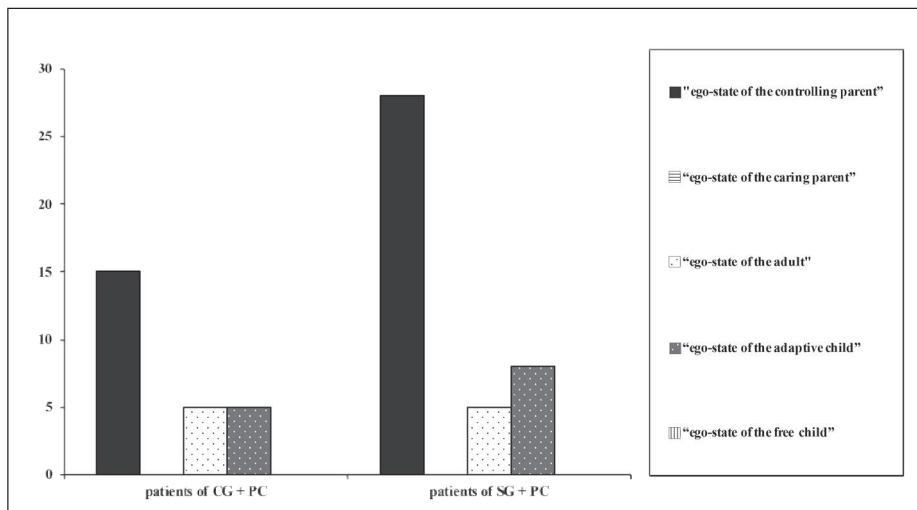


Fig. 1. Results of determining the dominant functional "ego-states" by the patients with paranoid schizophrenia under study.

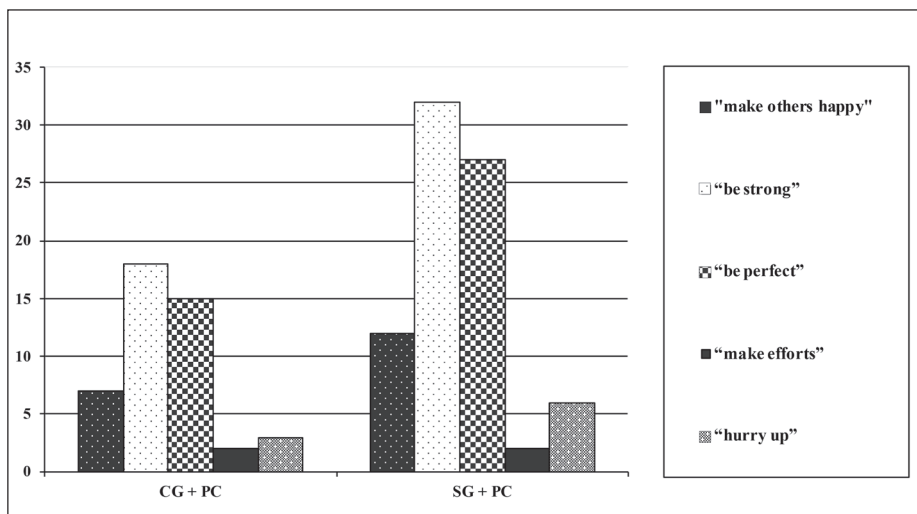


Fig. 2. Results of determining the leading "drivers".

in this group of the persons under study, the contamination of the "parent ego-state" and the disabled "child ego-state" are determined.

These individuals are inclined to criticize themselves from the "parent ego-state" and use the defense mechanism of "escape" in response to their "child ego-state". The more they "escape", the more the "parent ego-state" criticizes them. Thus, the individual is stuck in his/her negative feelings. This structure is formed on the early oral stage, when basic needs remain unsatisfied [8]. According to this hypothesis, their parents were excessively immersed in their own emotions, and children made an early decision not to speak about their needs in the "hope" that the parents would be satisfied and feel so good to take care of the children themselves [7].

Presence of the "adult ego-state" by the persons under study indicates a sufficient level of awareness of themselves and their rueful feelings. Thus, this group of patients has favorable prerequisites for conducting effective psychotherapy.

Thus, it is determined that the "ego-states of the adaptive child" and "controlling parent" dominate in the persons under study. The "ego-state of the caring parent" was not

defined by any patient. These data correlate with the results obtained in determining the dominant structural "ego-states". Any structural "ego-state" can manifest itself in any functional "ego-state", as a result of which the "parent ego-state" dominating in the structure of personality during regress manifests itself functionally in the "ego-state of the adaptive child" and "controlling parent" [6]. The functional "ego-state of the adaptive child" directly reflects the structure and provides adaptive behavior. Since the persons under study were subject to violence in childhood, a solution such as a "survival strategy" was expedient at that time, and this form of behavior was an opportunity to adapt to the environment in order to save the own life. But in the present time, dominance of the "ego state of the adaptive child" is no longer relevant and causes social maladjustment of these patients. The functional "ego-state of the controlling parent" directly reflects the parent introjects. The purpose of the "controlling parent" is to dominate, criticize, and devalue the others [9]. The persons under study absorbed these characteristics of their real parents and formed rigid introjects. "Ego-state of the caring parent" was not observed by any of the persons under study. This result can be explained by the fact that the persons under study did not receive enough care and support

from their parents and therefore did not form an introject of the positive “caring parent”. According to the clinical interview, the persons under study received 45% more physical negative manifestations of attention (for example, physical punishment) than positive ones (for example, kisses, hugs), and 60% more negative verbal manifestations of attention (for example, criticism) than positive verbal ones.

“Passive behavior as the escape from the real interaction” is the main way for such people to respond to the world around them. The resource for such people is awareness of their “escape” through action, which will result in restoration of the internal contact with the emotional sphere from which patients with schizophrenia are dissociated. Passive behavior is the manifestation of the process of dissociation – internal alienation from unpleasant emotions. They put much of their energy into their behavior. You must begin to act together with them to establish the contact. These individuals do not feel safe enough to demonstrate their needs and feelings. To get out of their typical state, they must involve their thinking process, but instead of thinking about their real needs and their satisfaction, they are inclined to fantasize a lot and replace reality with fantasy. Thinking is an internal process for them which they externalize a little. If you give positive support to such individuals, they begin to externalize their thinking process and come closer to reality.

The results that 3 patients (12%) of CG + PC and 5 (13%) patients of SG + PC make contact with the outside world through its understanding correlate with those obtained below about the leading “drivers”, namely with the dominance of the “driver” “be perfect”. For such individuals, manifestations of attention about the clarity of their thinking are important, and feelings are a zone of difficulty. To restore the contact with the feelings, they need to learn to test their ideas about the thoughts and feelings of others from the “ego-state of the caring parent”. These individuals do not feel safe enough and believe that they should control every detail, so they think everything over in detail, are skeptical about everything around. They often distort others as they project their own perception of danger.

Based on the results of the leading “drivers” determination, it is possible to determine that the behavioral pattern “be strong” plays a significant role in formation of the internal picture of the disease of the persons under study. It manifests itself by overly restrained behavior, alienation from the own feelings, confidence in the own strength and self possession. This “driver” ensures functioning of the personality after dissociation and enables the individual not to come into contact with his/her rueful feelings, which seem threatening and destructive to him/her. In order to “please” their parents, such individuals try to “be strong” without having needs and equating the concepts of “being strong” and “being good”. Their parents ignored their needs, as a result of which they made an early childhood decision “I would be really good if I didn’t have needs at all.” The driver “make others happy” is the manifestation of the functional “ego-state of the adaptive child” and provides adaptation behavior [10]. Thus, individuals who have been exposed to the traumatic influence of the parental figures

implement their old script decision to adapt in order to avoid additional negative incentives.

From 0 to 6 months the main task is “to be”. “Prohibitions” that a child can receive at this stage are “do not exist”, “do not feel”, “do not have needs”, “do not be healthy”, “do not be close” [11]. Patients with the paranoid schizophrenia are injured at the very early stage of their development, which causes manifestations at all levels of functioning. They are characterized by the basic mistrust, dissociation from their own experiences, disorientation in their own feelings and self-awareness. The basis of these experiences are two basic frustrated needs – in safety and in close contact. These individuals are afraid to let something go out of control and be “unprepared.” Such fear arises in early childhood, when a child in reality cannot protect itself from something unexpected happening. Thus, these individuals decided to control themselves, others and keep others at a safe distance.

The patients with “script prohibition” “do not be close” experienced negative manifestations of attention or neglect and rejection by their parents in childhood as a result of which they made the early childhood decision to “emotionally stay away from people in order to avoid negative feelings of abandonment, loneliness and anguish”. In order to avoid destructive criticism from their parents, they decided in their childhood “if I control everything, I can prevent all bad things and avoid danger” [11]. The “prohibition” “do not have your feelings” revealed by 67% of the examined patients of CG + PC and by 72% of SG + PC is tightly connected with the decision “do not be close” and it is an internal protection from pain. The “prohibition” “do not think” revealed by 55% patients of CG + PC and also by 48% of SG + PC, is formed from previous “bricks”: as a person is dissociated from the signals of his/her own body – sensations and feelings, his/her thoughts are based on his/her own fantasies, and not on real facts. This “prohibition” is manifested by excessive day-dreaming, inability to analyze data about the world around us and to distinguish our fantasies from reality. The “prohibition” “do not be healthy” revealed by 55% of the patients of CG + PC and by 61% of SG + PC can be explained that they materialized getting sick with schizophrenia.

For the “schizoid” the contamination of the “parental ego-state” is typical. A typical functional “ego-state” is the “ego-state of the adaptive child.” These features of functional and structural “ego-states” were identified by the patients under study. A characteristic “life position” is “I -, You -”, which corresponds to the criteria of the “personalized adaptation” of the “schizoid” and examination data [12]. The tone is usually smooth and monotonous, mimic and gesticulation are poor. Basic needs include recognition and satisfaction of their needs and feelings. People with such an adaptation avoid problem solution. Relevant “prohibitions” are “do not be healthy”, “do not be close”, “do not feel”, “do not think”, which correspond to the examination data. The “door for contacting” the “schizoid” are carried out through behavior, same was determined by the patients under study [13, 14].

For the “paranoic” the “parental contamination” and excluded “child’s ego-state” are typical, and the leading functional “ego-state” is the “ego-state of the controlling parent,” that corresponds to the data revealed in the patients. The “life position” is “I +, You -”, that is the second predominant among the patients. In the situations of significant stress it changes to the position “I -, You -”. The monotonous voice, straight and immovable posture are typical. The basic needs include trust and feeling safe. The main “prohibitions” are “do not feel”, “do not be close” and “do not trust”. The leading “directives” are “be perfect” and “be strong.” These characteristics coincide with the data obtained during the patients’ examination. The “door for contacting” the “paranoic” is thinking – understanding of thoughts, that leads to understanding of feelings and then to changes in behavior.

Thus, from the point of view of the transactional analysis theory, the schizophrenia-related disorders can be considered as a defensive reaction. Such a personality structure allows to dissociate the “intolerable” part of your own experience and “separate” it from yourself. The schizoid protection occurs when the others do not pay enough attention to the child’s needs or react negatively to any manifestation of his/her needs or feelings. As a result, these personalities form the basic existential position “I -, You -”, which is formed as a result of receiving such “prohibitions”: “do not have feelings, sensations and needs”, “do not think”, “do not be healthy”, “do not live”. The paranoid traits reflect the following aspects of the personality development: an episodic response to the child’s needs and the messages like “do not be close” and “do not trust”. As a result, he/she gets the idea that “if he/she controls everything, then he/she can prevent all the bad things from happening”.

This paper presents a theoretical generalization and practical optimization of the effectiveness of treatment and rehabilitation of patients with paranoid schizophrenia based on the study of features of clinical and psychopathological picture of the disease and the integration of transactional analysis into the complex treatment model to improve adherence to therapeutic recommendations, reduce the time frame of the inpatient phase of therapy and to provide the further reconstruction of the identity of the patient. Established scientific goals have been fully achieved, and the results of this study are completely original. A potential limitation of illustrating the effectiveness of this approach is the fact that, on the one hand, a longer stay of the patient in the hospital causes a significant reduction in the risk of re-hospitalization due to longer support of the patient with psychotherapy counseling, however, on the other hand, may be the trigger factor in the formation of the phenomenon of hospitalism.

CONCLUSIONS

Patients with paranoid schizophrenia do not trust anyone at an unconscious level (including their organs and thinking based on real facts) and are “forced” to build their own understanding of reality that would satisfy their

frustrated need for contact with an important close person (reliable affection pattern), because accepting of the fact that they were rejected by important close people and the awareness of their despair thereof is destructive for them. Thus, schizophrenia-related disorders can be considered as a defensive reaction. “Ego-states of the controlling parent” and “adaptive child” dominate in the patients with paranoid schizophrenia. The absence of the introject of the “ego-state of the caring parent” in the patients with paranoid schizophrenia is caused by a lack of care and support from parental figures in childhood. The patients with paranoid schizophrenia have the prevailing “life position” “I -, You -” as a reflection of basic distrust to the world around, devaluation of themselves and others. The leading “driver” “be strong” by the patients with paranoid schizophrenia provides functioning of the personality after dissociation. The “drivers” “make others happy” and “be strong” – dominating by the patients with paranoid schizophrenia are formed as a result of the psycho-traumatic influence of the “ego-state of the controlling parent” of parental figures and are functional manifestations of the “ego-state of the adaptive child”. According to the transactional analysis theory the patients under study did not pass the first stage of the personality development completely that violates the personality formation at the earliest stage and preconditions the pathological manifestations at all the functioning levels. The typical “script prohibitions” of the patients under study are “do not be close”, “do not feel”, “do not think” and “do not be healthy”. The characteristic “personalized adaptations” for the patients with paranoid schizophrenia are “schizoid” and “paranoic”.

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ORIGINAL ARTICLE

RETROSPECTIVE ANALYSIS OF LEPTOSPIROSIS MORBIDITY IN IVANO-FRANKIVSK REGION (EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS)

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ABSTRACT

The aim: To perform a retrospective analysis of leptospirosis morbidity in Ivano-Frankivsk region, to give the etiological characteristics of leptospirosis according to clinical-laboratory parameters, to determine the peculiarities of the course, prognostic features.

Materials and methods: Data of the State Enterprise "Ivano-Frankivsk Regional Laboratory Center of the Ministry of Health of Ukraine", reports and abstracts of medical records of the Regional Clinical Infectious Diseases Hospital for 2009–2018 were used. Clinical observation of patients, analysis of general-clinical, biochemical, and serological indices were carried out.

Results: The leptospirosis morbidity in Ivano-Frankivsk region during 2009–2018 was higher than in general in Ukraine. Activation of foci of leptospirosis *L. pomona* and grippotyphosa in 2009–2013, decrease of *L. grippotyphosa* and increase of *L. pomona* foci and "new" *L. autumnalis*, *australis*, *bataviae*, *cynopteri* in 2014–2018 were noted. The emergence of leptospirosis *L. autumnalis*, which had a high virulence and caused a severe course, was noted.

Conclusions: The leptospirosis morbidity in the Ivano-Frankivsk region over the past 10 years has exceeded the incidence in Ukraine and varied within 0.62–2.2 per 100,000. During the last 5 years, the serological structure has changed: *L. pomona* (27.7%) prevailed, *L. grippotyphosa* decreased (2.1%), *L. bataviae* and *cynopteri*, *autumnalis* increased (by 8.5%). Seasonality is shifted in the autumn–winter period (November–February) (*L. grippotyphosa*, *australis*, *pomona*, *canicola*). According to forecasts, leptospirosis induced by *L. autumnalis* was most adverse. Leptospiroses of serogroups *L. australis*, *bataviae*, *cynopteri* had a mild course, there were fewer complications.

KEY WORDS: Leptospirosis, seasonality, focus, serogroup, Ivano-Frankivsk region

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INTRODUCTION

The pandemic distribution of leptospirosis causes considerable attention to this disease. The disease is registered more than in 80 countries of the world and ranks third in terms of natural-focal diseases [1, 2, 3]. A lot of attention has been paid to the study of leptospirosis in Ukraine: epidemiology has been studied in different geographical areas, natural foci have been identified, and the link between morbidity and the farming system, clinical manifestations, treatment methods [4, 5] have been determined. The difficult economic situation in Ukraine led to the destruction of the system of sanitary-veterinary control of the population of rodents – the main sources of leptospirosis pathogens. Also, the etiological structure of morbidity, periodicity and seasonality has significantly changed [6]. Due to the severity of the clinical course and indicators of lethality, leptospirosis is one of the first places in infectious pathology. The mortality rate of the disease in Ukraine in recent years is about 10%. High mortality is associated with the toxic effect of the pathogen on the important organs of the human being (kidney, liver, heart) [3, 7, 8, 9].

Leptospirosis is the most widespread in the Western and Central regions of Ukraine, and the highest level is registered in the Lviv, Ivano-Frankivsk, Ternopil and Rivne

regions. The peculiarity of the Ivano-Frankivsk region is determined by its physical-geographical location, favorable weather conditions and composition of the fauna, which provides the prerequisites for the formation of the natural and anthropogenic foci of leptospirosis [5, 10]. Earlier in Ukraine and in the region the *Leptospira* of serovar *L. icterohaemorrhagiae* dominated, but from 2010 the leading place was occupied by *Leptospira L. grippotyphosa*, *L. pomona*, *L. canicola* and *L. hebdomadis* [5, 11]. It is believed that diseases caused by *Leptospira* of different serogroups have a single pathogenesis and pathological anatomy, clinical course, but this situation is not entirely accurate [12, 13]. Studies performed by us during 2009–2013 show the activation of the cells of *L. pomona* leptospirosis, as well as the occurrence of sporadic cases of *L. autumnalis* leptospirosis, which was characterized by high virulence and severity of the course of the disease. Some cases of leptospirosis of serogroups *L. australis* and *L. bataviae* had a mild course [14]. Taking into account the high morbidity, lethality of leptospirosis, the diversity of serovariants of pathogens, the emergence of new foci, more careful study of regional peculiarities of the epidemiological trend and clinical manifestations of this disease.

THE AIM

To perform a retrospective analysis of the morbidity of leptospirosis in the Ivano-Frankivsk region, to give the etiological characteristics of leptospirosis according to clinical-laboratory parameters, to determine the peculiarities of the course, prognostic signs.

MATERIALS AND METHODS

There were used data of the State Enterprise “Ivano-Frankivsk Regional Laboratory Center of the Ministry of Health of Ukraine”, reports of the Regional Clinical Infectious Diseases Hospital (RCIDH), hospitalization records of patients treated in RCIDH for the period from 2009 to 2018 inclusive. The clinical observation of patients, analysis of general-clinical and biochemical parameters, serological results of the examination were performed.

RESULTS AND DISCUSSION

The Ivano-Frankivsk region is located in the west of Ukraine and borders on Lviv, Ternopil, Chernivtsi and Zakarpatska. In the south it has a state border with Romania, where there is also high leptospirosis morbidity. By the nature of the relief, the region is divided into three parts: plain, foothill, and mountainous. The flat part – area near the river Dniester – is adjacent to the Dniester River. Here the forest-steppe dominates – the agricultural area of the region. In the middle part – the foothills – the forest landscape with broad-leaved forests prevails. The mountainous part of the region is occupied by the Eastern Carpathians. The region is in the zone of moderately continental climate, the most humid in Ukraine (humidity coefficient is 1.5-3.0). The average annual precipitation – is from 600 to 1550 mm. Water resources are represented by superficial (rivers, lakes, reservoirs) and underground waters. There are three water reservoirs in the region (Burshtyn, Chechva, Kniahynyn) with an area of 1670 hectares. The rivers with a length of up to 10 km – 8128, over 10 km – 188, and over 100 km – four, prevail: Dniester (1362 km), Prut (910 km), Limnytsa (122 km), Svicha (107 km). The prevalence of the population occupied by agricultural labor and geographical peculiarities contribute to the spread of pathogens of leptospirosis among rodents, animals and human infection.

The morbidity of leptospirosis in Ivano-Frankivsk oblast and in Ukraine over the last 10 years (2009-2018) is represented in Table I.

From the data in Table I, it is evident that the leptospirosis morbidity in the Ivano-Frankivsk region was higher than in the whole Ukraine in all years except 2012. The high rates were in 2010, 2014 and 2018 (2.71, 2.27, 2.26 per 100 thousand of population respectively).

Retrospective data on the incidence of leptospirosis in the region from 1980 to 2018 are represented in Figure 1.

Retrospectively, in the dynamics of morbidity in leptospirosis, three periods can be distinguished: from 1980 to 1988 – during some years the disease has not been registered or there have been sporadic cases. Since 1988,

the rise in the morbidity with a maximum in 1992 – 7.56 per 100 thousand of population, and a gradual decrease before 2000 – 2.34 per 100 thousand of population. Since then, the intensive index varies within the 3.48-0.62 per 100 thousand. Fluctuations indicate a certain periodicity of the increase in leptospirosis morbidity (3 years), which is related to the level of infection of domestic and agricultural animals, the number of rodents, the sanitary condition of settlements, the volume of deratization.

Territorial distribution of leptospirosis morbidity in the region has its own peculiarities. Primarily, the incidence was recorded the area near the Dniester river: Halych (2011 – 4.54 per 100 thousand), Kolomyia (2010 – 6.59 per 100 thousand), Dolyna (2012 – 2.92 per 100 thousand), Tysmenytsia (2013 – 2.32 per 100 thousand) areas. Gradually, the morbidity spread to the foothills where a dense river network is developed. In mountainous regions (Verkhovyna, Nadvirna), very rare cases were registered. The number of foci increases, there were added Tlumach (2016 – 4.12, 2018 – 6.29 per 100 thousand), Snyatyn (2016 – 3.15, 2018 – 10.76 per 100 thousand), Horodenka districts (2016 – 3.83, 2018 – 4.11 per 100 thousand). Over the past 5 years, mortality in the region was an average 0.32 per 100 thousand, in Ukraine – 0.12 per 100 thousand of population.

There is a clear summer-autumn seasonality of leptospirosis – from July to October. On average, the increase in morbidity begins to be recorded from July each year (9.2% from average annual) with a clear peak in September (24.6% from average annual) and October (10.8% from average annual). Seasonality is due to agricultural activity in summer (June-July) and harvesting in autumn (September), as well as infection through water. The second peak of morbidity is observed in December (12.3%), coincides with the migration of mouse rodents from the environment into the outbuildings.

In RCIDH over the past 10 years, 161 patients with leptospirosis were treated (85.7% of the diagnosis was confirmed serologically), 11 persons died (mortality rate – 6.8%). To evaluate changes in the etiological spectrum, patient charts for 2009-2013 and 2014-2018 were analyzed.

The basis of the etiological spectrum in the first group (63 serologically confirmed cases) were: *L. pomona* (22.92%) and *L. grippotyphosa* (22.92%), *L. icterohaemorrhagiae* (16.6%), *L. canicola* – 10.42 %, there were new *Leptospira* which were not previously registered: *L. autumnalis* (10.42%), *L. australis* (6.28%), *L. bataviae* (6.28%), *L. cynopteri* (4.16%).

In 2014-2018 (75 serologically confirmed cases), in the etiological composition of leptospirosis there was a shift. *L. pomona* also prevailed – 27.7%, but *L. grippotyphosa* decreased to 2.1%, *L. icterohaemorrhagiae* and *L. canicola* were often met (by 12.8%), *L. bataviae* and *L. cynopteri* *L. autumnalis* (by 8.5%) were registered more often, there were single cases of *L. tarassovi* and *L. australis* (by 2.1%). In part of patients in 2018 (15.9% of all), 2-3 *Leptospira* were detected in diagnostic titers (1:200-1:800). Among them the “new” *Leptospira* prevailed that were not pre-

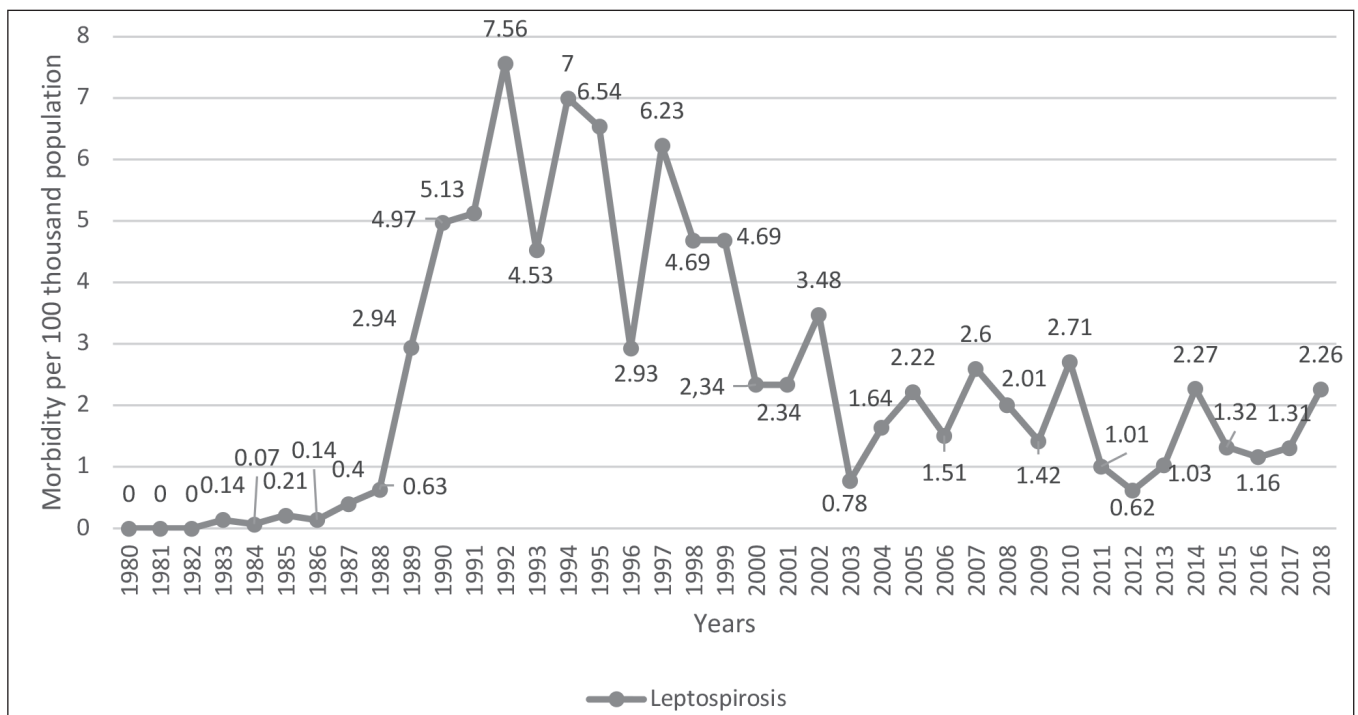


Fig. 1. Leptospirosis morbidity in Ivano-Frankivsk region for the period of 1980-2018 (per 100 thousand population)

Table I. Leptospirosis morbidity in Ivano-Frankivsk region and Ukraine for the period of 2009-2018 (per 100 thousand population).

Years	Ivano-Frankivsk region		Ukraine		Relative indicator of the comparison of the intensity index
	Absolute value	Per 100 thousand population	Absolute value	Per 100 thousand population	
2009	20	1.42	438	0.95	1.50
2010	38	2.71	632	1.37	1.98
2011	15	1.01	314	0.68	1.49
2012	9	0.62	316	0.7	-0.89
2013	14	1.03	320	0.79	1.30
2014	31	2.27	474	1.04	2.18
2015	18	1.32	301	0.7	1.89
2016	16	1.16	321	0.76	1.53
2017	18	1.31	322	0.78	1.68
2018	31	2.26	273	0.64	3.53

viously found – *L. javanica*, or were rarely registered – *L. bataviae*, *L. cynopteri*, *L. autumnalis*, often in conjunction with *L. icterohaemorrhagiae*. Of all the fatal cases in 2018, one is due to a combination of 3 serovariants: *L. icterohaemorrhagiae*, *L. grippityphosa* and *L. pomona*.

The patient charts (epidemiological history, clinical manifestations and laboratory data) were analyzed for the comparison of diseases caused by different serogroups. Men dominated – 77.1%, rural population, aged from 22 to 82, the largest group included individuals aged 50-70. Children were two under 18 years of age, with leptospirosis *L. rhomona*, of moderate severity. There were no mild forms, medium severe – 10.4% (*L. pomona*, *L. cynopteri*), anicteric – 4.3% (*L. grippityphosa*, *L. pomona*, *L. australis*). The rest of the patients were diagnosed with a severe form.

The water route predominated in summer in patients with *L. pomona*, *L. bataviae*, *L. grippityphosa*: patients drank dirty water, swam in the river, the pond. In other cases, the infection was associated with the care of animals and the contact with rodents. Patients were admitted to RCIH from June to October, but few cases – in the inter-season period in April-May (*L. grippityphosa*, *L. icterohaemorrhagiae*) and in the autumn-winter period (November-February) (*L. grippityphosa*, *L. australis*, *L. pomona*, *L. canicola*). It's interesting, that *L. autumnalis* corresponded to its name and was registered only in September-October.

Most patients had a high temperature – 39-40°C; it was somewhat lower in patients with *L. australis* – 37.5-39°C. All patients had pain in the muscles, except for a few patients with *L. australis*, *L. pomona*. Nausea and vomiting

were noted in 21% of patients, most often with *L. grippotyphosa* and *canicola*. All patients had jaundice, except for single anicteric forms. High average values of bilirubin were in patients with *L. icterohaemorrhagiae*, *autumnalis*, *canicola*, – 397.08 ± 25.20 , 485.89 ± 41.22 , 372.50 ± 30.09 $\mu\text{mol/l}$, respectively. The protein spectrum (total protein and albumins) changed most in *L. grippotyphosa* and *autumnalis* (decreased to 45 g/l and 40%, respectively). The least damage to the liver was in patients with *L. australis* and *cynopteri* (bilirubin – 108.36 ± 9.90 , 143.85 ± 12.45 $\mu\text{mol/l}$). The largest size of the liver was in patients with *L. autumnalis* (17.5 ± 0.8 cm), and the smallest one was in *L. canicola*, *grippotyphosa* (14.3 ± 0.1 cm). Anemia, leukocytosis, high ESR were in all patients, but *L. grippotyphosa* (leukocytosis – $26.0 \pm 1.8 \cdot 10^9$), *L. icterohaemorrhagiae* (Hb – 88 ± 6.2 g/l), *L. autumnalis* (ESR – 57.0 ± 4.2 mm/h) gave the greatest shifts in average hematological indices. In patients with *L. pomona*, *L. cynopteri*, *L. bataviae*, these indices changed to a much lesser extent.

Acute renal failure (ARF) with polyuria phenomena was present in almost all patients. The most severe ARF was caused by *L. icterohaemorrhagiae* (urea – 26.10 ± 3.83 mmol/l), *L. grippotyphosa* (creatinine – 375.79 ± 28.32 mmol/l), and also *L. autumnalis*, *canicola*. Significantly lower were those in patients with *L. cynopteri* and *australis* (urea – 17.98 ± 0 , creatinine – 173.55 ± 10.32 , urea – 19.08 ± 1.67 , creatinine – 277.90 ± 18.92 mmol/l, respectively). Acute hepatic encephalopathy (precoma I) and DIC syndrome were observed in 1 patient with *L. autumnalis* and in 1 patient with *L. grippotyphosa*. Infectious-toxic shock of the 1-2 degree was in 2 patients with *L. icterohaemorrhagiae* and in 1 patient with *L. canicola*. Hemorrhagic syndrome was most pronounced and more frequent in *L. grippotyphosa*, *icterohaemorrhagiae*, *autumnalis*. Reactive pancreatitis was observed in 76% of patients with different *Leptospira*, with the exception of *L. cynopteri*, *australis*, and was accompanied by an increase in the level of α -amylase of blood, diastase of urine, hyperglycemia. Pneumonia was found in 34.8% of patients with different serovariants, complicated by pleurisy, hydrothorax, respiratory insufficiency. Cardiac damage (carditis, atrial fibrillation) was observed in leptospiroses *L. icterohaemorrhagiae*, *grippotyphosa*, *autumnalis*, *pomona*. Unfortunately, *L. javanica* was registered only in combination with other *Leptospira*, that did not give us the possibility to reveal its clinical peculiarities.

Thus, in Ivano-Frankivsk oblast, the intensification of the foci of the leptospirosis of the serogroup *L. rhomona* and *grippotyphosa* in 2009-2013, the sharp reduction of *L. grippotyphosa* in 2014-2018 and the increase of the foci of *L. rhomona* and the “new” *Leptospira* *L. autumnalis*, *australis*, *bataviae*, *cynopteri*, *javanica*. The emergence of new foci of leptospirosis in foothill areas is explained by an increase in the number of infected rodents, mammals, domestic animals due to the infection of soil, water, unsatisfactory vaccination of domestic animals, the termination of both urban and field 2-time deratization. According to the clinical and laboratory features, one can distinguish etiologic differences

in leptospiroses, the occurrence of leptospirosis cases in the serogroup of *L. autumnalis*, which has high virulence, with the severity of the disease is not inferior to the traditional serogroups – *L. grippotyphosa* and *L. icterohaemorrhagiae*. Single cases of leptospirosis of serogroups *L. cynopteri* and *L. australis* have a mild course. These data require further investigation in terms of evidence.

CONCLUSIONS

1. The morbidity of leptospirosis in the Ivano-Frankivsk region over the past 10 years has exceeded the level of morbidity in Ukraine and ranged from 0.62 to 2.2 per 100 thousand of population.
2. During the last 5 years the serological structure of leptospiroses in the Ivano-Frankivsk oblast has changed. The leading role in epidemiology was *L. rhomona* (27.7%). The topicality of *L. grippotyphosa* (2.1%) has decreased, the registration of *L. bataviae* and *L. cynopteri*, *L. autumnalis* (by 8.5%) has increased.
3. Periodicity of rise in leptospirosis morbidity was determined every 3 years. Seasonality is shifted in the autumn-winter period (November-February) (*L. grippotyphosa*, *L. australis*, *L. pomona*, *L. canicola*).
4. Prognostic most adverse is the course of leptospirosis caused by *L. autumnalis*. Leptospiroses of the serogroups *L. australis*, *L. bataviae*, *L. cynopteri* had a relatively easier course and much less complications.

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ORIGINAL ARTICLE

THE EFFECT OF BETA-BLOCKERS ON A COURSE OF CHRONIC HEART FAILURE IN PATIENTS WITH A LOW TRIIODOTHYRONINE SYNDROME

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ABSTRACT

The aim: The aim is to study the effect of β -ABs in patients with LT_3S on the course of HF.

Materials and methods: 354 patients with HF on a background of post-infarction cardiosclerosis were included in the 2-year follow-up study. LT_3S was diagnosed at 89 (25.1%) patients. The levels of thyroid-stimulating hormone, free T_3 and T_4 , and reversible T_3 were determined. The echocardiography was performed.

Results: Patients with HF in combination with LT_3S have a heavier functional class by NYHA, greater dilatation of the left heart cavities, less myocardial contractility, a higher frequency of atrial fibrillation and re-hospitalization. The use of β -ABs in patients with HF without LT_3S leads to a likely decrease in hospitalization frequency, while in patients with LT_3S it has an opposite effect. The frequency of rehospitalization increases with an excess of β -ABs dose > 5 mg (equivalent to bisoprolol). At these patients a decrease in serum T_3 level and negative dynamics of parameters of intracardiac hemodynamics are observed.

Conclusions: The use of β -ABs in patients with LT_3S leads to an increase in re-hospitalization at a dose over 5.0 mg (equivalent to bisoprolol). In these patients there is a decrease in serum T_3 , an increase in T_4 level; and the ejection fraction decrease; and heart cavities size increase.

KEY WORDS: low triiodothyronine syndrome, β -blockers, thyroid-stimulating hormone, ROC curve, risk, ventricular dilatation, reversible triiodothyronine, heart failure

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INTRODUCTION

The progression of heart failure (HF) is a complex process affecting many organs and systems that leads to hormonal and immune alterations, including low triiodothyronine syndrome (low T_3 syndrome, LT_3S). The LT_3S is characterized by low levels of triiodothyronine (T_3), increased or normal reversible triiodothyronine (T_{3r}), normal or slightly altered levels of thyroid-stimulating hormone (TSH), and normal thyroxine (T_4) content, [1-3]. The occurrence of LT_3S in HF patients may reach 30% [3]. The LT_3S is associated with impaired peripheral conversion of inactive T_4 to active T_3 by deiodinases by increasing proinflammatory potential, hypoxia [4-8]. The LT_3S that accompanies HF can lead to a number of disorders, including the reduction of systolic heart function, the development of arrhythmias, increased vasoconstriction [1, 9-11]. The β -blockers (β -AB) reduce mortality (up to 30%) and morbidity in symptomatic patients with HF [12]. In addition, this group of medications did not show a significant effect on survival in decompensated patients. It is also known that β -ABs lead to blockade of deiodinases, which leads to a decrease in T_3 to T_4 conversion [13], thus they are prescribed to patients with hyperthyroidism. It may be supposed that the use of β -ABs in patients with HF in combination with LT_3S may increase its severity.

THE AIM

The aim is to study the effect of β -ABs in patients with LT_3S on the course of HF.

MATERIALS AND METHODS

The protocol of this prospective cohort study was approved by the local Ethics and Deontology Committee of Government Institution "L.T.Malaya Therapy National Institute" (Ukraine). All study procedures were performed in accordance with the ethical standards of the Declaration of Helsinki. From January 2015 to June 2019, we included patients in the study and conducted prospective follow-up (for 2 years). Patients were involved in the study on admission with the HF decompensation at the cardiology department. 354 patients with HF (110 women and 244 men) of the Caucasian race were included into the study. The inclusion criteria were: signed of the Patient Informed Consent, history of myocardial infarction (MI), verified diagnosis of HF II – IV functional classes (FC) by NYHA. The exclusion criteria: no informed consent, hemodynamically significant valvular heart defects, HF of other aetiology than cardiac infarction, hormone replacement therapy

Table I. Characteristics of groups of patients with HF according to presence /absence of the LT_3S (T_{3f} criterion ≤ 2.07 pmol / l)

Parameters	Groups of patients with HF (n = 354)		P
	Without LT_3S (n = 265)	With LT_3S (n = 89)	
1	2	3	4
Age, years	58,00 [54,00 – 68,00]	58,00 [54,00 – 66,00]	0,657
Male sex, n (%)	179 (67,5)	65 (73)	$\chi^2 = 0,936$ $p = 0,333$
IBM, kg/m ²	27,65 [25,71 – 31,15]	27,63 [25,52 – 31,74]	0,945
NYHA FC, n (%):			
I	124 (46,8)	18 (20,2)	$\chi^2 = 24,642$ $p = 0,0001$
II	123 (46,4)	54 (60,7)	
IV	18 (6,8)	17 (19,1)	
Angina pectoris FC, n (%):			
I	4 (1,5)	0	$\chi^2 = 2,143$ $p = 0,543$
II	29 (10,9)	10 (11,2)	
III	84 (31,7)	33 (37,1)	
Type 2 diabetes mellitus, n (%)	62 (23,8)	28 (31,8)	$\chi^2 = 2,236$ $p = 0,135$
Coronary intervention, n (%)	19 (7,2)	9 (10,1)	$\chi^2 = 0,792$ $p = 0,374$
Non-toxic goiter, n (%)	43 (16,2)	28 (31,5)	$\chi^2 = 9,644$ $p = 0,002$
Atrial fibrillation, n (%)	16 (6,0)	12 (13,5)	$\chi^2 = 5,070$ $p = 0,024$
β -AB, n (%)	208 (78,8)	74 (86,0)	$\chi^2 = 2,183$ $p = 0,140$
- Carvedilol, n (%)	22 (10,6)	16 (21,6)	$\chi^2 = 5,711$ $p = 0,017$
- Bisoprolol, n (%)	186 (89,4)	58 (78,4)	
ACEI, n (%)	225 (84,9)	81 (91,0)	$\chi^2 = 2,119$ $p = 0,146$
ARB, n (%)	37 (14,0)	6 (6,7)	$\chi^2 = 3,255$ $p = 0,072$
MRA, n (%)	125 (47,2)	40 (44,9)	$\chi^2 = 0,133$ $p = 0,716$
Diuretics, n (%)	223 (84,2)	70 (78,7)	$\chi^2 = 1,413$ $p = 0,235$
Ivabradine, n (%)	4 (1,5)	1 (1,1)	$\chi^2 = 0,071$ $p = 0,790$
Digoxin, n (%)	60 (22,6)	19 (21,3)	$\chi^2 = 0,064$ $p = 0,800$
SBP, mm Hg	147,00 [130,00 – 160,00]	145,00 [130,00 – 160,00]	0,854
DBP, mm Hg	90,00 [80,00 – 100,00]	90,00 [80,00 – 100,00]	0,944
HR, min ⁻¹	74,00 [66,50 – 82,00]	75,00 [66,00 – 84,00]	0,613
RBC, 10 ¹² / l	4,50 [4,10 – 4,82]	4,60 [4,30 – 4,86]	0,194
Hb, g/l	140,00 [128,00 – 150,00]	141,00 [134,00 – 150,00]	0,194
WBC, 10 ⁹ / l	6,30 [5,50 – 7,42]	6,60 [5,40 – 7,40]	0,717
Granulocytes, %	60,30 [54,78 – 66,70]	60,15 [55,65 – 64,43]	0,923
Lymphocytes, %	30,00 [24,60 – 36,70]	31,40 [26,55 – 35,05]	0,367
Monocytes, %	6,70 [5,10 – 8,00]	6,70 [5,10 – 8,25]	0,912
Platelets, 10 ⁹ / l	201,00 [172,25 – 239,00]	207,00 [178,50 – 239,75]	0,408
Creatinine, μ mol / l	90,40 [77,00 – 105,80]	92,50 [78,50 – 103,75]	0,899
Glucose, mmol / l	5,47 [4,80 – 6,51]	5,72 [4,93 – 6,73]	0,377
Total cholesterol, mmol / l	4,71 [3,77 – 5,70]	5,48 [3,77 – 5,91]	0,753
LDL, mmol / l	2,56 [1,87 – 3,48]	2,90 [1,80 – 3,75]	0,208
HDL, mmol / l	1,10 [0,95 – 1,37]	1,00 [0,88 – 1,25]	0,030
VLDL, mmol / l	0,69 [0,46 – 1,07]	0,77 [0,52 – 1,06]	0,326
TG, mmol / l	1,49 [1,03 – 2,26]	1,63 [1,08 – 2,21]	0,635
TSH, mIU/l	1,60 [1,05 – 2,50]	1,36 [0,91 – 2,05]	0,035
T_{3p} , pmol/l	2,87 [2,44 – 3,53]	1,72 [1,51 – 1,87]	0,0001
T_{4p} , pmol/l	14,24 [11,11 – 16,52]	14,89 [12,46 – 16,32]	0,308
T_{3u} , pg/ml	289,54 [214,53 – 367,77]	252,26 [205,55 – 309,75]	0,041
T_{3u} / T_{4u} ratio	0,20 [0,16 – 0,29]	0,12 [0,09 – 0,14]	0,0001
LV EDD, cm	5,40 [5,00 – 5,80]	5,60 [5,20 – 6,00]	0,020
LV EDV, ml	143,15 [119,86 – 168,62]	155,61 [131,23 – 182,17]	0,059
LV ESD, cm	4,07 [3,70 – 4,61]	4,40 [4,00 – 4,93]	0,0001
LV ESV, ml	74,09 [59,10 – 99,23]	89,00 [71,11 – 115,743]	0,0001
IVS, cm	1,20 [1,10 – 1,22]	1,20 [1,10 – 1,21]	0,792
LVPW, cm	1,20 [1,10 – 1,20]	1,15 [1,10 – 1,20]	0,925
LV EF, %	45,97 [37,22 – 55,17]	38,19 [32,18 – 48,05]	0,0001
LV MM, g	317,30 [280,83 – 367,51]	336,07 [302,03 – 378,31]	0,037
LV IMM, g/m ²	109,48 [96,20 – 125,32]	114,77 [99,37 – 129,80]	0,307
LA, cm	4,20 [3,90 – 4,50]	4,10 [3,80 – 4,40]	0,255
ILA, cm/m ²	0,46 [0,41 – 0,50]	0,48 [0,44 – 0,54]	0,006
RV, cm	2,70 [2,58 – 3,00]	2,80 [2,60 – 3,00]	0,302
RA, cm	3,70 [3,50 – 4,00]	3,60 [3,50 – 3,98]	0,523

Notes: SBP – Systolic blood pressure; DBP – diastolic blood pressure, HR – heart rate, TG – triacylglycerides, ACEI – angiotensin-converting-enzyme inhibitors, ARB – angiotensin II receptor blockers, MRA – Mineralocorticoid receptor antagonists.

with L-thyroxine, thyro-suppressive treatment, clinical or subclinical hypothyroidism, pathothyroidism, inflammatory diseases, other serious pathologies (neoplasm, tuberculosis), which could complicate treatment or reduce life expectancy. Diagnosis of HF and treatment of patients were performed according to the recommendations of the European Society of Cardiologists [14].

For patients doppler echocardiography was performed using the VIVID-3 ultrasonic diagnostic system (General Electric, USA). End-diastolic and end-systolic dimensions (EDD and ESD respectively) of the left ventricle (LV), the thickness of the interventricular septum (IVS), the LV posterior wall (LVPW), the diameter of the left atrium (LA), right ventricle (RV), other options were determined. The left ventricle end-diastolic volume (LV EDV) and the end-systolic volume (LV ESV), the ejection fraction (LVEF) of the left ventricle, the left atrium index (ILA), the mass of the LV myocardium (MM LV) and its index (IMM LV) were calculated.

To determine blood serum levels of thyroid-stimulating hormone (TSH) (normal range 0.3–4.0 mIU / l), free T_3 (T_{3f}) (normal range 2.5–5.8 pmol / l) and free T_4 (T_{4f}) (normal range – 10–25 pmol / l) reagent kits (ELISA “TSH”, “ T_{4f} ” and “ T_{3f} ” by Hema, Ukraine) were used. The level of reversible triiodothyronine (T_{3r}) (normal range – 90–350 pg / ml) was determined using an ELISA kit reagent (Elab-science®, China). Immunoferment studies were performed on a semi-automatic enzyme-linked immunosorbent analyser “Immunochem-2100” (High technology, USA). During the biennial follow-up period the course of HF was evaluated, the frequency of repeated hospitalizations for decompensation of the disease, mortality, the dynamics of thyroid hormones levels and parameters of intracardiac hemodynamics were studied.

The analysis of the normality of the distribution of indicators was performed using the Shapiro – Wilk test. Continuous variables are given as the median (Me) and the interquartile range (for non-normally distributed variables) or as the mean (M) and the standard deviation ($\pm$ SD) (in the case of the normal distribution). The quantitative indicators were compared using a non-parametric criterion – Mann – Whitney and a paired T-test (in the study of parameter dynamics). The categorical variables were compared using the Pearson's chi-squared test (χ^2) (Yates correction for traits less than 10). The receiver operating characteristic (ROC) curves were analysed to determine the prognostic level of T_{3f} . The relative risk (RR) of re-hospitalization and mortality was calculated with a 95% confidence interval (CI). P-value less than 0.05 was considered as statistically significant. Statistical processing was performed using IBM® SPSS® Statistics, 20.0 (free-download full Version) and MedCalc, 18.9.1 (free version) software packages.

RESULTS

Previously we reported that the threshold level of T_{3f} for the low T3 syndrome below the lower threshold of its normal

range (2.5 pmol / l) did not demonstrate a relationship of this state with peculiarities of the HF course. The threshold value was reduced to ≤ 2.07 pmol / l and the effect of LT_3S on HF course was studied [15].

At the initial stage of the statistical analysis, all patients with HF were divided into 2 groups: the first group included 265 (74.9%) patients who had normal levels of T_{3f} , T_{4f} and TSH (Table I); in the second – 89 (25.1%) patients with T_{3f} level ≤ 2.07 pmol / l at normal T_{4f} and TSH levels. These patients were diagnosed with LT_3S .

In the group of patients with HF in combination with LT_3S II FC by NYHA was in 20.2% of patients, compared to 46.8% in the group without this syndrome, III FC by NYHA was in 60.7% of patients, versus 46.4% in another group and IV FC was in 19.1% of patients, versus 6.8% in the group without this syndrome ($\chi^2 = 24.642$; $p = 0.0001$); these patients more often had non-toxic goiter (31.5% versus 16.2%, ($\chi^2 = 9.644$; $p < 0.002$) and atrial fibrillation (13.5% vs. 6.0%, ($\chi^2 = 5.070$; $p < 0.024$) (Table I).

The patients with LT_3S compared with patients without it, had lower HDL (by 9.1%, $p = 0.030$); TSH (by 15.0%, $p = 0.035$), T_{3f} (by 36.6%, $p = 0.0001$), T_{3r} (by 12.9%, $p = 0.041$) levels, and T_{3f} / T_{4f} ratio (by 40.0%, $p = 0.0001$); higher LV EDD (by 3.7%, $p = 0.020$), LV ESD (by 7.2%, $p = 0.0001$), LV ESV (by 20.1%, $p = 0.0001$), MM (by 5.9%, $p = 0.037$) and ILA (by 4.3%, $p = 0.006$); lower LV EF value (by 7.9%, $p = 0.0001$) (see Table I).

The patients in both groups did not differ in HF therapy. However, patients with LT_3S were more likely to take carvedilol (21.6% vs. 10.6%, $p = 0.017$), comparing to patients of another group.

The hospitalization frequency due to HF decompensation within 2 years, among patients with LT_3S , was higher (55.1%, vs. 20.4%, $p = 0.0001$).

The relative risk of re-hospitalization of HF patients with LT_3S within 2 years was 2.098 [1.506 – 2.921] ($p = 0.0001$). The relative risk of death of these patients during the specified period, was 1.642 [0.711 – 3.797] ($p = 0.245$). Thus, the presence of LT_3S significantly increased the relative risk of re-hospitalization within 2 years from 20.4 to 55.1% (see Table II).

The effect of the β -ABs use on the frequency of rehospitalization due to HF decompensation was analyzed (Table III).

In the group of patients with HF without LT_3S , the frequency of re-hospitalization was lower when using beta-blockers, compared to that in patients who could not take this group of medication due to side effects (16.3% vs. 35.1%, respectively, $p = 0.002$). The relative risk of re-hospitalization in this group when using β -blockers is 0.466 [0.291 – 0.744] ($p = 0.0014$). Thus, the use of β -blockers in the group of patients with HF without LT_3S leads to a 53.6% reduction in the relative risk of re-hospitalization within 2 years.

In the group of patients with HF, flowing on the background of LT_3S , taking β -AB, there is a tendency to increase the frequency of re-hospitalization, compared with that in patients who were unable to take this group

Table II. The Biennial rehospitalisation and mortality.

Parameters	Groups of patients with HF (n = 354)		χ^2 (p)
	Without LT ₃ S (n = 265)	With LT ₃ S (n = 89)	
Rehospitalisation, n (%)	54 (20,4)	49 (55,1)	38,838 (0,0001)
Mortality, n (%)	14 (5,3)	8 (9,0)	1,570 (0,210)

Table III. β -Abs effect on biennial rehospitalization and mortality

Parameters	All patients with HF (n = 354)		χ^2 (p)
	Without β -ABs (n = 72)	With β -ABs (n = 282)	
Rehospitalization, n (%)	25 (34,7)	78 (27,7)	1,387 (0,239)
Mortality, n (%)	6 (8,3)	16 (5,7)	0,694 (0,405)
Patientes with HF without LT ₃ S (n = 265)			
	Without β -ABs (n = 57)	With β -ABs (n = 208)	
Rehospitalization, n (%)	20 (35,1)	34 (16,3)	9,685 (0,002)
Mortality, n (%)	3 (5,3)	11 (5,3)	0,107* (0,744)
Patientes with HF with LT ₃ S (n = 89)			
	Without β -ABs (n = 15)	With β -ABs (n = 74)	
Rehospitalization, n (%)	5 (33,3)	44 (59,5)	3,440 (0,064)
Mortality, n (%)	3 (20,0)	5 (6,8)	1,300* (0,255)

Note * χ^2 Yates

Table IV. Dependence of frequency of hospitalization and mortality on type of β -AB

Parameters	Patients with HF without LT ₃ S (n = 265)		χ^2 (p)
	Carvedilol (n = 22)	Bisoprolol (n = 186)	
Rehospitalization, n (%)	8 (36,4)	26 (14,0)	7,201 (0,007)
Mortality, n (%)	0	11 (5,9)	1,374 (0,241)
Patients with HF and LT ₃ S (n = 89)			
	Carvedilol (n = 16)	Bisoprolol (n = 58)	
Rehospitalization, n (%)	11 (68,8)	33 (56,9)	0,731 (0,393)
Mortality, n (%)	2 (12,5)	3 (5,2)	1,069 (0,301)

of drugs (59.5%, vs. 33.3 %, respectively, $p = 0,064$) (see Table. 3). The relative risk of re-hospitalization in the LT₃S group when using β -ABs was 2.378 [1,088 – 5,199] ($p = 0.029$). Thus, the use of β -blockers in patients with HF in combination with LT₃S significantly increases the risk of re-hospitalization from 33.3% to 59.5%.

In the group of patients with HF without LT₃S, 22 patients received carvedilol and 186 – bisoprolol. The incidence of re-hospitalization in the bisoprolol subgroup (14.0%) was significantly lower, compared to that with carvedilol (36.4) ($p = 0.007$). No significant difference in mortality was found.

In the LT₃S group, 16 patients were treated with carvedilol and 58 with bisoprolol. No significant difference was found in the effect of carvedilol, compared with bisoprolol, on the frequency of hospitalization and mortality in patients with LT₃S (see Table IV).

Taking into account small subgroups of patients with LT₃S taking bisoprolol or carvedilol, for further analysis a

unifying dose of these drugs was performed according to the scheme presented in [16].

A ROC analysis was performed to stratify the dose of β -ABs affecting the risk of re-hospitalization in patients with HF. The incidence of hospitalization in the group of patients with HF without LT₃S was found to be higher at a dose of ≤ 5.0 mg (equivalent to bisoprolol), comparing to a higher dose (sensitivity – 85.00% and specificity – 47.41%, $p = 0,0001$).

At the same time, ROC analysis showed the opposite effect of β -ABs in the group of patients with LT₃S. The re-hospitalization incidence of patients with HF in combination with LT₃S increases when the optimal cut-off value for the β -ABs dose exceeds 5 mg (equivalent to bisoprolol) (sensitivity – 67.35% and specificity – 57.50%, $p = 0.025$) (see Table V).

Among all patients, the biennial hospitalization frequency when using β -ABs at a dose less or equal to 5 mg was 33.5%, compared with 23.7% when receiving over 5 mg

Table V. Relationship of β -ABs dose (equivalent to bisoprolol) with rehospitalization (ROC analysis)

Group	Cut-off value	Area under the curve (AUC)	95% CI	Sensitivity, %	Specificity, %	p
All patients with HF (n = 354)	$\leq 2,5$ mg	0,549	0,498 - 0,600	35,78	73,90	0,131
HF without LT ₃ S (n = 265)	$\leq 5,0$ mg	0,686	0,630 - 0,739	85,00	47,41	0,0001
HF with LT ₃ S (n = 89)	$> 5,0$ mg	0,634	0,525 - 0,733	67,35	57,50	0,025

Table VI. Dependence of re-hospitalization incidence on dose of β -ABs (n (%))

All patients with HF (n = 354)		χ^2 (p)
≤ 5 mg (n = 194)	> 5 mg (n = 160)	
65 (33,5)	38 (23,7)	4,033 (0,045)
Patients with HF without LT ₃ S (n = 265)		
≤ 5 mg (n = 155)	> 5 mg (n = 110)	
49 (31,6)	5 (4,5)	29,053 (0,0001)
Patients with HF and LT ₃ S (n = 89)		
≤ 5 mg (n = 39)	> 5 mg (n = 50)	
16 (41,0)	33 (60,0)	5,523 (0,019)

($p = 0.045$) (Table IV). The relative risk of re-hospitalization with β -ABs dose exceeding 5 mg was 0.709 [0.504 – 0.977] ($p = 0.048$). It can be concluded that the use of β -ABs in such dose at HF (without LT₃S) reduces the relative risk of re-hospitalization by 29.3 %.

Among patients with HF without LT₃S, the biennial re-hospitalization incidence when using β -ABs at a dose less or equal to 5 mg was 31.6%, compared with 4.5% when receiving over 5 mg ($p = 0.0001$).

The relative risk of re-hospitalization of these patients using dose of β -blockers over 5 mg was 0.144 [0.059 – 0.349] ($p = 0.0001$). Thus, the use of β -ABs > 5 mg in the group of patients with HF without LT₃S reduces the relative risk of re-hospitalization.

In the group of patients with HF in combination with LT₃S, the biennial re-hospitalization incidence when using β -ABs at a dose of less or equal to 5 mg was 41.0%, compared to 60.0% when receiving over 5 mg ($p = 0.019$) (see Table VI). The relative risk of re-hospitalization in patients with LT₃S taken β -ABs > 5 mg was 1.609 [1,051 – 2,462] ($p = 0.029$). Thus, the use of β -ABs > 5 mg in the group of patients with HF in combination with LT₃S increases the relative risk of re-hospitalization by 46.3%.

In order to study the effect of different doses of β -ABs on the dynamics of parameters of intra-cardiac hemodynamics and thyroid hormones levels for 2 years, patients with HF and LT₃S were divided into 2 groups. The first group included 39 (43.8%) patients who failed to increase the dose of β -ABs over 5 mg due to side effect. The second group included 50 (56.2%) patients taking β -blockers at a dose over 5 mg. In the group of patients with LT₃S with a dose of β -ABs below and equal to 5 mg for 2 years, there was an increase in T_{3f} level (by 0.185 ± 0.413 pmol / l, $p =$

0.011), a decrease in ESD (by 0.480 ± 1.079 cm, $p = 0.008$) and ESV (20.734 ± 6.984 ml, $p = 0.029$), an increase in LV EF value (by $10.053 \pm 13.370\%$, $p = 0.0001$); decrease in LA (0.262 ± 0.719 cm, $p = 0.035$) and RV size (0.208 ± 0.372 cm, $p = 0.010$).

In addition, in the subgroup of patients with LT₃S receiving β -ABs at a dose exceeding 5 mg / day during 2 years a decrease in serum T_{3f} level (by 0.107 ± 0.275 pmol / l, $p = 0.014$), an increase of the T_{4f} level (by $1,148 \pm 2,245$ pmol / l, $p = 0.002$), the decrease in the value of the T_{3f} / T_{4f} ratio (by 0.018 ± 0.035 , $p = 0.002$); increase in LV EDD (by 0.350 ± 0.813 cm, $p = 0.004$), LV EDV (by 24.800 ± 5.14 ml, $p = 0.003$), LV ESD (by 0.342 ± 1.045 cm, $p = 0.003$), LV ESV (by 21.325 ± 48.750 ml, $p = 0.004$), ILA (0.947 ± 0.221 cm, $p = 0.0001$), and RV (0.419 ± 0.586 cm, $p = 0.0001$) were observed.

DISCUSSION

The thyroid secretes several hormones, including T₄, T₃, and T_{3f}, and it is the sole source of T₄. Unlike the latter, T₃ (biologically more active hormone) is secreted by the thyroid gland not more than 20% of the total level. The remaining T₃ is synthesized by other tissues (outside the thyroid gland) by enzymatically removing the iodine atom from the T₄ molecule by deiodinases, which exist in several forms. Type I deiodinase (D₁) is primarily found in the liver and kidneys and is responsible for the synthesis of 80% of T₃. Type II deiodinase (D₂) is mostly located in the brain and muscles, including the human heart, and regulates T₃ tissue concentration. Type III (D₃) deiodinase, converting T₄ to reversible T₃ (inactive), decreases serum T_{3f} level [17]. T_{3f} penetrates the membrane into the cell and is responsible

Table VII. Dependence of thyroid hormone dynamics and intracardiac haemodynamic parameters on dose of β -ABs in patients with HF in combination with LT₃S (n = 89).

Parameters	Dose of β -ABs	
	≤ 5 mg (n = 39)	> 5 mg (n = 50)
1	2	3
TSH, mIU/l	+ 0,027 $\pm$ 0,809 (p = 0,833)	+ 0,155 $\pm$ 0,895 (p = 0,259)
T _{3f} pmol/l	+ 0,185 $\pm$ 0,413 (p = 0,011)	- 0,107 $\pm$ 0,275 (p = 0,014)
T _{4f} pmol/l	+ 0,539 $\pm$ 2,926 (p = 0,276)	+ 1,148 $\pm$ 2,245 (p = 0,002)
T _{3f} / T _{4f} ratio	- 0,0002 $\pm$ 0,0607 (p = 0,980)	- 0,018 $\pm$ 0,035 (p = 0,002)
LV EDD, cm	- 0,221 $\pm$ 1,045 (p = 0,194)	+ 0,350 $\pm$ 0,813 (p = 0,004)
LV EDV, ml	-13,249 $\pm$ 73,454 (p = 0,267)	+ 24,800 $\pm$ 5,140 (p = 0,003)
LV ESD, cm	- 0,480 $\pm$ 1,079 (p = 0,008)	+ 0,342 $\pm$ 1,045 (p = 0,026)
LV ESV, ml	- 20,734 $\pm$ 6,984 (p = 0,029)	+ 21,325 $\pm$ 48,750 (p = 0,004)
LV EF, %	+ 10,053 $\pm$ 13,370 (p = 0,0001)	- 3,245 $\pm$ 17,367 (p = 0,197)
LA, cm	- 0,262 $\pm$ 0,719 (p = 0,035)	+ 0,060 $\pm$ 0,657 (p = 0,526)
ILA, cm/m ²	+ 0,911 $\pm$ 0,183 (p = 0,0001)	+ 0,947 $\pm$ 0,221 (p = 0,0001)
RV, cm	- 0,208 $\pm$ 0,372 (p = 0,010)	+ 0,419 $\pm$ 0,586 (p = 0,0001)
RA, cm	- 0,165 $\pm$ 0,619 (p = 0,214)	+ 0,067 $\pm$ 0,649 (p = 0,515)

for genomic and non-genomic effects. T₃ has a positive inotropic and chronotropic effect on the myocardium, regulating the transcription of myocyte-specific genes [17]. T₃ directly affects vascular unstriated muscles, contributing to dilatation of arterioles and reduced peripheral vascular resistance. For normal functioning of the cardiovascular system, optimal concentrations of thyroid hormones are necessary; their excess or deficiency have a disintegrating effect. A decrease in serum T₃ concentration and a parallel increase in T_{3r} are a common result of many diseases, such as injuries, starvation and post-surgery conditions. [18] Such changes in function of "Hypothalamic-pituitary-thyroid" axis, are generally called as LT₃S. In the fasting state, this transition from the production of the metabolically potent hormone T₃ to the synthesis of the metabolic-inactive inverse (reversible) T_{3r} plays a compensatory role. However, in chronic conditions, such as HF and depression, low T₃ concentration can cause adverse effects [18]. The main mechanism of low serum T₃ concentration in patients with nontyroid disease is reduced D₁ activity in the liver. Increased concentrations of cytokines, such as interleukin-6 and tumor necrosis factor α , cause violation of hepatic D₁ expression. Other mechanisms involved in the pathogenesis of low T₃ syndrome include a decrease in the concentration of thyroid hormone binding proteins and a decrease in the secretion of thyroid hormone and TSH. Dopamine secretion and prolonged hypercortisolemia may play a role in this process [18]. There is no consensus in the literature regarding the criteria for defining low T₃ syndrome. So in [19, 20] a lower value of the range of the norm T_{3p}, which ranged from 4.0 to 2.5 pmol / l, at normal levels of T_{4f} and TSH are used. In the Articles of the Italian researchers [21, 22] new ones in addition to the above mentioned parameters, an additional criterion – an

increased T_{3r} level is introduced. In the previous work, we were unable to form a representative group of patients with HF with decreased T_{3f} levels, normal TSH and T_{4f} concentrations, and increased T_{3r} levels [15]. It has also been demonstrated that the risk of re-hospitalization of patients with HF with regard to decompensation of the disease increases when reaching the cut-off value for serum T_{3f} concentration below or equal to 2.07 pmol / l [15]. In our study, the incidence of LT₃S (T_{3f} $\leq$ 2.07 pmol / l) in patients with HF is 25.1%.

According to Japanese researchers, even patients with HF of I FC by NYHA and in the stage of compensation have some early manifestations of LT₃S [19]. In patients with HF, this syndrome is associated with poor LV function, tachyarrhythmia and increased mortality [23]. A low T₃ concentration in HF is a stronger prognostic predictor than dyslipidemia, age, or LV EF [23]. We revealed that in the group HF in combination with the LT₃S patients have a more severe NYHA functional class, they have more often atrial fibrillation, more severe dilatation of cavities of the left parts of the heart and less contractile capacity of myocardium, higher incidence of rehospitalization due to HF decompensation during two years.

Clinical trial results showed that β -ABs has a positive effect on the clinical course of HF and reduces the risk of five-year mortality by 30% [12, 24, 25]. In Recommendations of the European Society of Cardiologists (ESC) [14] and the American College of Cardiologists and the American Association of Cardiologists (ACC / ANA) [26] it is emphasized that β -ABs are a first-line drug for the treatment of patients with HF. In addition, β -ABs has also long been used in the treatment of symptomatic patients with thyrotoxicosis. This drugs block metabolism of thyroid hormones by inhibiting D₁ outside the glandula [13]. This effect is inherent in the

non-selective β -ABs [27], but it is also characteristic of selective medication [28, 29]. In patients a decrease in serum T_3 concentration [30, 31] and an increase in T_{3f} levels by inhibiting its breakdown are observed. Usually, patients remain euthyroid and have stable serum TSH values [29]. But in patients with low thyroid hormones levels (including LT_3S) at the start of β -ABs treatment, this phenomenon may have clinical implications in the long term [32]. Clinical manifestations of β -ABs withdrawal are also associated with sharp fluctuations in thyroid levels [33, 34].

During the statistical analysis, we revealed that the use of β -ABs in patients with HF without LT_3S leads to a likely reduction in re-hospitalization within 2 years. At the same time, in the presence of LT_3S β -blockers led to an increase in the frequency of re-hospitalization of patients due to HF decompensation. Using ROC analysis, it was found out that the incidence of re-hospitalization in the group of patients with HF without LT_3S was higher at a dose of β -ABs below and equal to 5.0 mg (equivalent to bisoprolol), compared to a higher dose. The reverse effect of β -ABs was detected in the group of patients with LT_3S . The risk of re-hospitalization of patients with HF in combination with low T_3 syndrome increases with an excess of β -blockers dose over 5 mg. In the subgroup of patients receiving β -ABs at a dose over 5 mg / day, within 2 years a decrease in serum T_{3f} level and the T_{3f} / T_{4f} ratio, an increase in T_{4f} level, further enlargement of the heart cavities and reduction of LV EF were observed. This may indicate further growth affect of low triiodothyronine syndrome.

Thus, it is likely that at the beginning of the treatment by β -ABs in patients with HF it is necessary to exclude the presence of LT_3S . It is not advisable to titrate a dose of β -ABs higher than 5 mg (equivalent to bisoprolol) in patients with HF in combination with LT_3S .

Our research has strengths and weaknesses. The advantage is the study protocol and very strict inclusion criteria. Unlike other studies, this approach has allowed the creation of a very homogeneous group with the exclusion of patients with sub- and clinical thyroid dysfunction, with concomitant pathology that may affect hormone levels. On the other hand, such criteria did not allow the formation of a large group of patients with LT_3S who received β -ABs with different selectivity to receptors. Also, our study is limited to 2 years of follow-up period.

CONCLUSIONS

The incidence of low triiodothyronine syndrome in patients with heart failure is 25.1%. Patients in this category have more severe NYHA functional class, more frequent atrial fibrillation, greater dilatation of the left heart cavities and less myocardial contractility, a higher frequency of re-hospitalization for HF decompensation over two years. The use of β -blockers in patients with heart failure without low triiodothyronine syndrome leads to a likely reduction in hospitalization. At the same time, the use of β -blockers in patients with low triiodothyronine syndrome results in an increased incidence of re-hospitalization. The re-hospitalization incidence in the group of patients with

heart failure without low T_3 syndrome is higher at a dose of ≤ 5.0 mg (equivalent to bisoprolol), compared to a higher dose. The effect of β -blockers is reversed in low triiodothyronine syndrome: for this patients the risk of re-hospitalization for HF decompensation when the dose of β -blockers is over 5 mg; a decrease in serum triiodothyronine level and in T_{3f} / T_{4f} ratio, an increase in thyroxine; further enlargement of the heart cavities and reduction of the ejection fraction are observed.

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ORIGINAL ARTICLE

SOME CLINICAL AND EPIDEMIOLOGICAL FEATURES OF INFLUENZA-ASSOCIATED PNEUMONIA DEPENDING ON THE ETIOLOGICAL AGENT

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ABSTRACT

The aim: To analyze the epidemiological data and clinical course of pneumonia as a complication of influenza in the Poltava region, depending on the etiological agent.

Materials and methods: We have analysed the data of the official reporting documentation provided by the State Institution "Poltava Regional Laboratory Center of the Ministry of Health of Ukraine" and the chart of patients with a laboratory confirmed diagnosis of influenza who were in inpatient treatment at the Poltava Regional Clinical Infectious Hospital (PRCIH) in 2016–2018 years. In more detail, we examined 95 patients with pneumonia, which complicated the course of the flu. There were 43 (45.26%) female patients, — 52 (54.74%) male patients, aged 18 to 80 years.

Results: Studies have shown that despite a decrease in the incidence of influenza in recent years, the incidence of pneumonia, which complicated the course of the flu, remained consistently high (19.7% – 20.8%) with bacteriological isolation of *S.pneumoniae* (22.11%), *S.aureus* (13.68%), *Haemophilus influenza* (4.21%) and *E.coli* (3.16%). Severe course of pneumonia with bloody sputum, evident shortness of breath, bilateral lung damage, and need for oxygen support were significantly more frequently reported in patients with isolated *S.pneumoniae* and *S.aureus*. However, the severe course of the disease with the formation of abscesses in the lungs was observed only in the group *S.aureus* despite the relatively young age of such patients and significantly fewer risk factors for severe influenza and comorbidities.

Conclusions: The study showed a consistently high incidence of pneumonia, which complicated the flu and caused by *S.pneumoniae*, *S.aureus*, *Haemophilus influenza* and *E.coli*. Pneumonia caused by *S.pneumoniae* and *S.aureus*, were characterized the most severe course; however, a severe course with the formation of abscesses in the lung tissue was observed only in the group of *S.aureus*.

KEY WORDS: influenza-associated pneumonia, etiological structure, risk factors, clinical features

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INTRODUCTION

Influenza is the most common and widespread infectious disease on the globe. Thus, according to estimates, influenza and acute respiratory diseases (ARD) annually affects from 4 to 15% of the world's population, and 250-450 thousand people die from influenza and its complications [1]. Incidence of disease increases to 50-70% during the influenza pandemics, which are associated with genetic shift in the pathogen in the human population. Moreover, mortality from influenza and its complications ranks first among all infectious diseases [2, 3, 4, 5]

Pneumonia remains the main cause of morbidity, hospitalization and mortality in an influenza, and therefore it is a very complicated health problem worldwide, including developed countries. It is known that the influenza is complicated with pneumonia in 10-20% of all patients with influenza, and in about half of the hospitalized patients [6, 7]. According to present knowledge, there is a tendency for a severe course of community-acquired pneumonia (CAP) that occurs with influenza [2]. Often such pneumonia is characterized by severe intoxication syndrome, hemodynamic disorders, severe respiratory failure with the development of acute respiratory distress syndrome (ARDS).

In recent years, reports in the scientific literature indicate that the etiology of pneumonia has expanded significantly, and among the agents that can cause severe pneumonia, there may be various associations of influenza virus with bacteria [4].

At the same time, only some reports describe the dependence of the severity of pneumonia on the etiological factor and attempt to distinguish the characteristic clinical features of pneumonia caused by a particular pathogen or a combination thereof.

Thus, the problem of influenza-associated pneumonia is topical, which makes the need for further study of the features of their clinical course, generalization of the results of laboratory and instrumental and radiological studies to optimize diagnostic and therapeutic tactics taking into account the etiological structure.

THE AIM

To analyze the epidemiological data and clinical course of pneumonia as a complication of influenza in the Poltava region, depending on the etiological factor.

MATERIALS AND METHODS

To achieve this aim we have analysed the data of the official reporting documentation provided by the State Institution "Poltava Regional Laboratory Center of the Ministry of Health of Ukraine" and the chart of patients with a laboratory confirmed diagnosis of influenza who were in inpatient treatment at the Poltava Regional Clinical Infectious Hospital (PRCIH) in 2016-2018. In more detail, we examined 95 patients with pneumonia, which complicated the course of the flu. There were 43 (45.26%) female patients, – 52 (54.74%) male patients, aged 18 to 80 years.

Influenza was diagnosed based on epidemiological, clinical data, serological findings with the determination of the increasing of specific antibody titers in the hemagglutination inhibition reaction with influenza diagnostic agent A/H1N1, A/H2N2, A/H3N2, B and the detection of Influenza virus A/H1N1, A/H2N2, A/H3N2, B in polymerase chain reaction (PCR). Among the factors that complicated the course of the disease, we considered obesity, chronic pathology of the cardiovascular and respiratory systems, diabetes mellitus, immunodeficiency of various etiology and neoplasms.

The diagnosis of pneumonia was established on the basis of a comprehensive analysis of epidemiological and clinical and laboratory data with mandatory X-ray/tomographic confirmation. The course of pneumonia and the need for hospitalization to the intensive care unit was determined on the IDSA/ATS (American Thoracic Society and Infectious Diseases Society of America), wherein we were guided by the order of the Ministry of Health of Ukraine from March 19, 2007 No. 128, and classified the patients into one of five groups, depending on the severity and the presence of concomitant pathology. Treatment of patients with pneumonia I-III group was performed in the diagnostic department of PRCIH; IV – in the intensive care unit. All patients received antiviral therapy (oseltamivir 150 mg/day in the case of pneumonia of II-III group, and 300 mg/day in the case of pneumonia of IV group), antibiotic therapy in accordance with the Protocol of treatment orders of the Ministry of Health of Ukraine from May 20, 2009, No. 189, and from November 18, 2009, No. 832), oxygen therapy for indications.

We have conducted a comprehensive examination of all patients with determination of pulse oxygen saturation (SpO_2), bacteriological examination of sputum, laboratory and additional tests depending on concomitant and diagnosed pathology with the involvement of consultants of the corresponding profile.

For statistical processing of the research results were used parametric and non-parametric methods of variational statistics. The difference was considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The conducted studies showed that 1344 cases of influenza were registered in 2016 according to the official reporting documentation provided by the State Institution "Poltava Regional Laboratory Center of the Ministry of Health of

Ukraine", in Poltava Region, which amounted to 93.91 cases per 100 thousand population, and 305892 cases of acute infections of the upper respiratory tract with multiple or indeterminate localization (21374.46 cases per 100 thousand population). In 2017, the influenza has been diagnosed in 216 patients in the Poltava region (15.22 cases per 100 thousand population), and acute infections of the upper respiratory tract with multiple or uncertain localizations have been diagnosed in 232050 patients (16353.32 cases per 100 thousand population). In 2018, the diagnosis of "Influenza" was established in 359 patients of Poltava region (25.53 cases per 100 thousand population), and the diagnosis of acute infections of the upper respiratory tract with multiple or uncertain localizations – in 224905 patients (15996.19 cases per 100 thousand population). We also observed a similar tendency to reduce the incidence of influenza in recent years according to the data of PRCIH, as indirectly indicated by the number of hospitalized patients with this pathology. Thus, in 2016, 499 patients were treated in PRCIH with the diagnosis of "Influenza" (65 of them had influenza A, 2 had influenza B, and 432 were diagnosed with influenza clinically with clear clinical and epidemiological data), then in 2017, there were only 104 patients with influenza (influenza A – in 25 patients, influenza B – in 3 patients, influenza (clinically) – in 76 patients), and in 2018 – 187 patients (influenza A – 32, influenza B – 27, influenza (clinically) – 128). It should be noted that the incidence rate has not shown significant growth in recent years, but the frequency of pneumonia, which complicated the influenza according to the PRCIH data, remained at a stable high level (20.84% in 2016, 20.19% in 2017 and 19.7% in 2018).

Among patients with pneumonia, in a microbiological examination of sputum a pathogenic bacterial agent was identified in 41 (43.16%) patients. We isolated the following microorganisms: *S.pneumoniae* – 21 patients, *S.aureus* – 13, *Haemophilus influenza* – 4 and *E.coli* – 3 patients.

It should also be noted that in 2 patients we isolated *Streptococcus viridans*, and in one patient – *Staphylococcus epidermidis* and in another one – *Candida spp.* However, according to the literature, the development of bronchopulmonary inflammation for these microorganisms is not characteristic. Their isolation from sputum is likely to indicate the contamination of the material by the flora of the upper respiratory tract, not the etiological significance of these microorganisms.

Most patients with pneumonia as a complication of the influenza ($n = 61$; 64.21%) were over 40 years of age. However, we have noticed a trend that patients who develop pneumonia caused by *S.aureus*, were younger – 23 to 49 years (mean age 37.46 ± 2.44 years). Whereas in the group in which we were not able to isolate the bacterial pathogen in sputum, the average age was 49.58 ± 2.22 years (23 to 80 years), in the group of *S.pneumoniae* – 57.85 ± 2.89 years (28 to 76 years), in the group of *Haemophilus influenza* – 51.50 ± 7.45 years (33 to 69 years) and in the group of *E.coli* – 48.33 ± 7.05 years (35 to 59 years).

In general, the risk factors for severe influenza and,

accordingly, the development of pneumonia were identified with the following frequency: chronic cardiovascular diseases – 42.10% (n = 40), disorders of the respiratory system – 36.84% (n = 35), obesity – 7.37% (n = 7), diabetes mellitus – 8.42% (n = 8), liver cirrhosis – 2.10% (n = 2), long smoking period – 41.05% (n = 39), HIV infection – 1.05% (n = 1), neoplasms – 2.10% (n = 2). (Table I). Interestingly, the frequency of identifying risk factors for severe influenza and concomitant pathology was essentially the same when comparing groups with different bacterial agents and the group in which we were not able to isolate the bacterial pathogen in sputum, except that in the group of *S.aureus* risk factors and concomitant pathology were diagnosed significantly less frequently. This was probably due to the fact that the patients were in the group *S.aureus* were younger.

The analysis of the time of admission of patients to the hospital showed that early hospitalization prevailed among the patients. This fact indirectly indicated a serious course of the disease and possible early development of complications. Almost half of the patients – 42 (44.21%) were hospitalized for 1-3 days. The bacterial pathogen was not isolated in sputum substantially in all of these patients, which may indicate the primary viral nature of possible complications. Almost the same number of patients (n = 47; 49.47%) were hospitalized for 4-6 days, 6 (6.32%) patients were hospitalized after 6 days, with no characteristic differences between groups of patients with different bacterial agents in sputum.

At the pre-admission phase, treatment was prescribed to 54 (56.84%) patients: 28 patients received antibacterial therapy drugs, 26 received antiviral therapy drugs, which, as a rule, were combined with symptomatic agents.

In most patients, the disease was characterized by an acute onset with a predominance of symptoms of general toxic syndrome with such frequency: hyperthermia (n = 94; 98.95%), headache (n = 65; 68.42%), muscle pain (n = 37; 38.95%). Moreover, 11 (11.59%) patients experienced symptoms of severe toxic syndrome, such as nausea and vomiting. Catarrhal syndrome of varying severity was also observed in the vast majority of patients: scleritis and hyperemia of the oropharyngeal mucosa (n = 63; 66.32%), dry (n = 61; 64.21%) and productive (n = 30; 31.58%) cough. The dry cough changed on productive in most patients in the dynamics (n = 85; 89.47%). The type of sputum was mucoid – (n = 60; 63.16%), mucopurulent – (n = 26; 27.37%) or bloody – (n = 9; 9.47%). The high incidence of chest pain (n = 38; 40.00%) and shortness of breath (n = 32; 33.67%) should be noted. Thus, in general, the clinical course at the onset of the disease was typical substantially in all patients with the prevalence of manifestations of toxic syndrome without differences between groups of patients with different bacterial agents in sputum, except that bloody sputum and severe breathlessness were observed only in groups *S. pneumoniae* (n = 7 and n = 20, respectively) or *S.aureus* (n = 2 and n = 12, respectively).

Analysis of physical changes in the lungs in hospitalized patients showed that signs of pneumonia (dullness on per-

cussion sound over the affected part of the lung, weakened or bronchial breathing, small bubbling rales, crepitations) were determined in almost all patients (n=93; 97.89%).

Of all patients, 10 (10.53%) were assigned to Clinical Group IV, among them unilateral inflammatory process in lungs was observed only in 1 patient (with *S.pneumoniae*), in the other 9 patients (4 patients with *S.pneumoniae*, 3 patients with *S.aureus* and 2 patients without isolated bacterial agent) the inflammation was bilateral. Clinical Group III included 39 (41.05%) patients: 36 patients had bilateral and 3 patients had unilateral pneumonia. Clinical group II included 46 (48.42%) patients; all except one had unilateral lung injury.

Radiographic picture of pneumonia in patients had certain features. Unilateral (n = 49; 51.58%) or bilateral inflammatory process (n = 46; 48.42%) was recorded essentially with the same frequency. It should be noted that bilateral lesions were mainly observed in patients with *S.pneumoniae* (n = 17), *S.aureus* (n = 11), or in a group of patients without bacterial agent (n = 18) and assumed primary viral cause of inflammatory changes in the lungs.

However, in case of primary viral interstitial inflammation, we observed a moderate compaction of the lung tissue with signs of diffuse bronchiolitis and bronchopneumonia (a symptom of “ground-glass”, “tree-and-bud”) with small centrilobular foci without decay and rarely with consolidation phenomena. Whereas, as usually with the addition of the bacterial flora, the inflammatory process began on the periphery and in the lower lobes, first in the form of an increased pulmonary vascularity and inhomogeneous nodular opacity, which later spread in severe cases and formed massive confluent subtotal or total infiltrative changes in the lungs. In some patients, on the background of such massive infiltrative changes, microabscesses were formed in the lung tissue. Interestingly, in all of these patients with abscesses (n = 4), we isolated *S.aureus* in sputum. The roots of the lungs were substantially expanded and infiltrated. By means of an estimation of the x-ray pattern in the dynamics, it was found that the resorption of infiltrates lasted more than 12 days, and residual pneumonia was preserved in the overwhelming majority (n = 84; 88.42%) of the persons discharged from the hospital.

The level of SpO_2 was significantly differed depending on the prevalence of inflammatory process in the lungs in patients. In patients with pneumonia of Clinical Group II – III, values of SpO_2 ranged from 99% to 91% (mean $95.1 \pm 0.3\%$) without oxygen inhalation. In the case of pneumonia of Clinical Group IV with bilateral lung injury, blood oxygen saturation varied from 92 to 60% without inhalation of oxygen. Depending on the etiological factor, these were the patients with *S.pneumoniae* (n = 5), *S.aureus* (n = 3), or without any bacterial agent (n = 2) with a possible primary viral nature of the disease. All patients with pneumonia of Clinical Group IV received treatment in the intensive care unit to improve the condition from 4 to 14 days (mean – 6.3 ± 1.2 days).

In recent years, the scientific literature has reported a severe course severe course and high mortality of pneumonia,

Table I. Demographic indicators and the frequency of the risk factors of the complicated course of influenza in the examined patients with pneumonia

		Total		No bact. agent		S.pneumoniae		S.aureus		Haemophilus influenza		E.coli		Other pathogens	
		abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Gender	male	52	54.74	27	54.00	12	57.14	8	61.54	2	50.00	2	66.67	2	50.00
	female	43	45.26	23	46.00	9	42.86	5	38.46	2	50.00	1	33.33	2	50.00
Age	18-30	16	16.84	12	24.00	2	9.52	2	15.38	-	-	-	-	-	-
	31-40	18	18.95	4	8.00	3	14.29	8	61.54	1	25.00	1	33.33	1	25.00
	41-50	19	20.00	7	14.00	5	23.81	3	23.08	1	25.00	1	33.33	2	50.00
	51-60	27	28.42	20	40.00	4	19.05	-	-	1	25.00	1	33.33	1	25.00
	61 and older	15	15.79	7	14.00	7	33.33	-	-	1	25.00	-	-	-	-
	pathology of the respiratory system	35	36.84	21	42.00	11	52.38	1	7.69*	1	25.00	-	-	1	25.00
Risk Factors	pathology of the cardiovascular system	40	42.11	23	46.00	13	61.90	2	15.38*	1	25.00	1	33.33	-	-
	obesity	7	7.37	5	10.00	2	9.52	-	-	-	-	-	-	-	-
	diabetes mellitus	8	8.42	5	10.00	2	9.52	-	-	-	-	-	-	1	25.00
	hepatic cirrhosis	2	2.11	1	2.00	1	4.76	-	-	-	-	-	-	-	-
	smoking	39	41.05	16	32.00	11	52.38	7	53.85	2	50.00	1	33.33	2	50.00
	HIV infection	1	1.05	1	2.00	-	-	-	-	-	-	-	-	-	-
	malignant neoplasms	2	2.11	1	2.00	1	4.76	-	-	-	-	-	-	-	-

Note. Percentages were calculated from the number of patients in the appropriate group, depending on the etiologic agent

* - significant difference compared to the patients in the group *S.pneumoniae* and group without bacterial agent ($p < 0.05$)

which is caused by a combination of *S.aureus* and influenza virus. This fact is associated with a powerful role of staphylococcus virulence factors in the pathogenesis: Pantone-Valentine leukocidin, phenol-soluble modulins $\alpha 1$ (PSM $\alpha 1$) and $\alpha 3$ (PSM $\alpha 3$), α -hemolysin (Hla), etc. [8, 9, 10, 11]. We have identified an association *S.aureus* and influenza virus in 13 (13.68%) patients. The clinical symptomatology of pneumonia in these individuals was mainly characterized by a severe course, despite the relatively young age of the patients. Almost every fourth of these patients (3 out of 13) was assigned to Clinical Group IV. Moreover, vast majority of patients with *S.aureus* was characterized by bilateral subtotal or total lung injury (11 of 13).

In the course of our research we aimed to analyze the epidemiological data and clinical course of pneumonia as a complication of influenza in the Poltava region, depending on the etiologic factor. We have received original data for our region and consider that the results of our research can be extrapolated to the whole country, taking into account the geographical and socio-economic situation of the region. On the other hand, this may be an insignificant methodological limitation of the results obtained. However,

the above data confirm the trends described in the literature that require further research to optimize the diagnostic and therapeutic tactics of pneumonia which complicate influenza, depending on their etiologic agent.

CONCLUSIONS

Studies have shown that despite a decrease in the incidence of influenza in recent years, the incidence of pneumonia, which complicated the course of influenza, remained consistently high (19.7% – 20.8%) with bacteriological isolation of *S.pneumoniae* (22.11%), *S.aureus* (13.68%), *Haemophilus influenza* (4.21%) and *E.coli* (3.16%). Severe course of pneumonia with bloody sputum, evident shortness of breath, bilateral lung damage, and need for oxygen support were significantly more frequently reported in patients with isolated *S.pneumoniae* and *S.aureus*. However, the severe course of the disease with the formation of abscesses in the lungs was observed only in the group *S.aureus* despite the relatively young age of such patients and significantly fewer risk factors for severe influenza and comorbidities.

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ORIGINAL ARTICLE

ANTIBIOTIC RESISTANCE OF THE NASOPHARYNX MICROBIOTA IN PATIENTS WITH INFLAMMATORY PROCESSES

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ABSTRACT

The aim: To study the species composition of microorganisms isolated from patients with inflammatory processes of different localization and to monitor the sensitivity of isolated microorganisms to the most commonly used antibiotics.

Materials and methods: During 2017–2019, we examined 517 patients with acute inflammatory processes of the nasopharynx. To establish the etiological structure of pathogens of inflammatory processes, a microbiological study of nasopharyngeal washes was carried out using classical methods of isolation and identification of microorganisms. The sampling of the test material from patients was performed during the first visit to a doctor, before the appointment and implementation of etiopathogenetic therapy. To study the sensitivity of isolated microorganisms to antibacterial drugs, we used the method of paper disks (manufactured by "Pharmaktiv" Ltd. (Kyiv, Ukraine) and Himedia (India).

Results and conclusions: The microflora of the mucous membranes of the nose and throat in patients with acute inflammatory processes of the nasopharynx is represented mainly by monoisolates of *staphylococci* and *streptococci*. The sensitivity of isolated microorganisms to antibiotics varied between different types of microorganisms and between different antibiotics. Cefazolin showed efficacy against all types of microorganisms. *K. pneumonia* strains showed the highest antibiotic resistance. Among the gram-positive cocci, 18 strains (4.2%) resistant to all antibiotics were isolated, which can be regarded as the circulation of hospital strains outside the medical institutions.

KEY WORDS: antibiotics, resistance, microflora, nose, and throat

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INTRODUCTION

Despite significant achievements in medicine, infectious diseases remain one of the leading causes of morbidity and mortality worldwide [1]. According to the statistical reports of the Sanitary and Epidemiological Service, over the past 20 years of observation, infectious diseases have affected from 7 to 10 million residents of Ukraine annually. The share of childhood infections in the overall morbidity structure has a steady upward trend (from 49.1% in 1995 to 65.4% in 2013) with a significant increase in both viral and bacterial infections [2]. The only option of etiotropic therapy that can curb the infectious process, prevent the development of complications and contribute to the rapid health improvement of patients [3] is the use of antibiotics. It is noted that their use has increased by 40% worldwide over the last decade [4]. However, in his article L. L. Founou [5] noted that antibiotics have become «endangered species» at risk due to the rapid spread of antibiotic resistance worldwide [6]. It is known that genes responsible for the formation of resistance can enter the bacterial population from commensals (non-pathogenic representatives) and from microorganisms living in the environment. Therefore, to understand the mechanisms of acquisition and spread of antibiotic resistance among pathogenic bacteria fully, it is necessary to study the entire microbiota carefully [1]. Scientific advances in recent years have significantly

deepened the knowledge about the biological properties of pathogens, their genetic characteristics, adaptive variability, the ability to persist on biotic and abiotic objects in the composition of biofilms, and to create associations of pathogenic, conditionally pathogenic bacteria with representatives of normofloor bacteria [7]. However, the problems of diagnosis and treatment of purulent-inflammatory diseases remain relevant and diverse. An important step in the prevention of complications and chronicity of purulent-inflammatory processes is the monitoring of the species composition and antibiotic resistance of microorganisms that are released from patients and from epidemiologically significant objects of the internal environment of medical institutions.

THE AIM

To study the species composition of microorganisms isolated from patients with inflammatory processes of different localization and to monitor the sensitivity of isolated microorganisms to the most commonly used antibiotics.

MANUALS AND METHODS

During 2017–2019, we examined 517 patients with acute inflammatory processes of the nasopharynx, 350 (pharynx)

Table 1. Specific structure of pathogens of purulent-inflammatory processes of patients with acute inflammatory processes of the nasopharynx

Kind of microorganism	The number of isolated microorganisms					
	from the throat (n = 401)		from the nose (n = 167)		Totally (n = 568)	
	abs	%	abs	%	abs	%
<i>S. aureus</i>	98	24,5	100	59,9	198	34,9
<i>S. pyogenes</i>	129	32,2	54	32,3	183	32,2
<i>Candida spp.</i>	64	15,9	-	0	64	11,3
<i>K. pneumoniae</i>	57	14,2	-	0	57	10,0
<i>S. haemoliticus</i>	43	10,7	13	7,8	56	9,9
<i>K. ozaenae</i>	7	1,7	-	0	7	1,2
<i>Moraxella spp.</i>	3	0,8	-	0	3	0,5

Table 2. The sensitivity of isolated microorganisms to antibiotics.

Kind of microorganism	The relative number of strains (%) sensitive to antibiotics							
	ciprofloxacin	oxacillin	ceftriaxone	Amoxicillin	cefazolin	azithromycin	clarithromycin	erythromycin
<i>S. aureus</i> , n=198	56,0±0,3	56,1±0,3	72,2±0,3	45,9±0,6	62,6±0,4	42,9±0,6	54,1±0,3	45,9±0,3
<i>S. haemoliticus</i> , n=43	58,7±1,1	53,5±1,1	84,0±0,9	80,0±0,9	65,1±1,1	66,7±1,1	76,7±1,0	27,9±1,0
<i>S. pyogenes</i> , n=183	74,9±0,3	46,4±0,6	63,4±0,4	33,3±0,8	49,2±0,6	78,7±0,3	48,6±0,6	42,1±0,6
<i>K. pneumoniae</i> , n=57	37,9±0,9	10,0±0,5	11,6±0,6	9,3±0,5	41,9±0,9	8,5±0,5	5,4±0,4	7,0±0,4
<i>K. ozaenae</i> , n=7	14,3±5,8	-	0	28,6±7,5	71,4±7,5	57,1±8,2	-	-
<i>Moraxella spp.</i> , n=3	33,3±23,6	33,3±23,6	33,3±23,6	0	66,7±23,6	0	-	-

and 167 (nose). Patients underwent comprehensive examination in accordance with standards of care. The examination procedure for these individuals was conducted in accordance with the standards of the ethics committee. To establish the etiological structure of pathogens of inflammatory processes, a microbiological study of nasopharyngeal washes was carried out on the basis of the microbiological laboratory of Sumy State University using classical methods of isolation and identification of microorganisms. The sampling of the test material from patients was performed during the first visit to a doctor, before the appointment and implementation of etiopathogenetic therapy. To study the sensitivity of isolated microorganisms to antibacterial drugs, we used the method of paper disks (manufactured by "Pharmaktiv" Ltd. (Kyiv, Ukraine) and Himedia (India). The results of the studies were subjected to statistical analysis. Graph Pad Quik Calcs was used to calculate the t-Student test.

RESULTS

On the basis of clinical and epidemiological data, the following diagnoses were previously established for patients: 43.1% of tonsillitis cases; 15.7% – sore throat;

32.3% – rhinosinusitis; 8.9% are adenoids, rhinopharyngitis. In total, 401 strains of microorganisms from the pharynx and 167 from the nose were isolated and identified. The frequency of isolation of microorganisms in a monoculture from the pharynx was 90.9% (318 isolates), from the nose – 100% (167 strains). Microbial associations were isolated in 32 patients (9.1% of patients examined) from the throat. The specific structure of pathogens of purulent-inflammatory processes is presented in table 1.

When studying the frequency of occurrence of various taxa of microorganisms in a clinically significant amount ($\geq 10^6$ CFU / ml of wash from a swab) in the studied biotopes, it was found that microorganisms isolated from the mucous membrane of the pharynx were represented by 5 genera, from the nasal mucosa – 3 genera (Table 1).

In the general structure of the species spectrum, *S. aureus* took the first place (34.9%) and was dominant in the material from the nose (59.9%). It should be noted that *S. aureus* is considered a transient representative of the microflora of the nose and this result indicates a significant spread in the population of carriers of pathogenic *staphylococcus*. In the frequency of discharge from the mucous membrane, the first place was taken by a representative of the genus *Streptococcus* – *S. pyogenes* (32.2%). The obtained result

Table 3. Resistance profiles of isolated microorganisms

Kind of microorganisms, quantity	Profile of antibiotic resistance	The relative number of polyresistant strains, %
<i>S. aureus</i> , n=198	Z□□OA□E□	19,1
	□C□□AK□□	6,0
	□CI□AK□□	4,0
	ZC□OAKEF	5,6
	Z□I□A□□□	3,0
	ZC□□□□□F	2,5
	□□I□A□□F	2,0
	□□□A□□□F	1,5
<i>S. haemoliticus</i> , n=43	□□IO□□E□	11,6
	□□□O□□EF	9,3
	□□I□AK□□	6,9
	ZC□OAKEF	4,7
<i>S. pyogenes</i> , n=183	□C□OA□E□	10,9
	□□I□AK□□	1,1
	□C□OAE□	4,4
	□C□OAK□□	2,7
	□□□OAKEF	3,3
	ZC□OAKEF	2,7
	Z□I□A□□□	3,3
	□CI□A□□□	1,1
<i>K. pneumonia</i> , n=57	□C□OA□□□	14,0
	ZC□OAK□□	14,0
	□□□OAKEF	15,8
	Z□I□A□E□	5,3

makes it possible to re-evaluate the pathogenic potential of this microorganism, since it is a representative of the normal microflora of the respiratory tract and under certain conditions can cause purulent-inflammatory processes in the respiratory tract. *Candida* (15.9%) was on the third place according to the frequency of excretion. The fourth was *Klebsiella pneumoniae* (14.2%). Analyzing the results of microbiological examination of the pharyngeal material from patients with ENT pathology, it should be noted that in all cases of isolation of associations of microorganisms were observed 2 or 3 component fungal-bacterial complexes. In 13 (3.7%) patients, *S. aureus* and *Sandida spp.*; in 16 (4.6%) – *S. aureus*, *K. pneumonia* and *Sandida spp.*, in 3 (0.85%) – *S. pyogenes* and *Sandida spp.*

Our results differ from the data of Krasii N.I. [7], who primarily selected representatives of the Enterobacteriaceae family ($59.7 \pm 2.2\%$) from the oropharynx of patients from the Department of Anesthesiology and Intensive Care, while *staphylococci* were only ($10.6 \pm 1.4\%$). This difference is probably due to the fact that the mucous membranes of the oropharynx of patients in the intensive care unit were colonized by nosocomial strains, and in our study,

mainly conditionally pathogenic representatives of the autochthonous microflora of the nose and pharynx were isolated from the patients.

The need for studying the species composition of microbiota and for exploring the sensitivity of microorganisms to antibiotics is considered a key link in effective therapy of infectious pathology. However, in practice doctors are more likely to perform empirical antibiotic therapy relying on the choice of drugs on world and local antibiotic resistance data. Therefore, constant monitoring of the species composition of the microbiota and its sensitivity to antibacterial drugs should be carried out at all levels – international, national, regional and local. Although the sensitivity of microorganisms to antibiotics varies greatly in different countries, regions, and even medical institutions, there is an idea of a group of problem microorganisms (ESKAPE – *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, whose *Pseudomonas aeruginosa* and *Enterobacter* are studied) which take significantly more attention. Our results indicate a known prevalence among patients with inflammatory processes of the nasopharynx of some representatives from the ESKAPE

group, which once again confirms the need for a thorough study of their antibiogram. When studying sensitivity to antibiotics, we selected antibiotics with different mechanisms of action, and therefore, different genetic mechanisms are usually involved in the formation of resistance to them. The results of a study of the sensitivity of isolated microorganisms to antibiotics are shown in table 2.

As can be seen from the above (Table 2), the most active antibiotic against all tested microorganisms is cefazolin, to which were sensitive from 41.9% to 71.4% of microorganisms. Among the bacteria of the strain *K. pneumoniae* were most often resistant to different mechanism of action of antibiotics (from 95.6 to 58.2% resistant isolates). Particular attention is drawn to the isolation of a large number of strains with multiple antibiotic resistance – 49.1% of *K. pneumoniae* isolates, 32.6% of *S. haemolyticus* isolates, 36.9% of *S. aureus* isolates, 30.6% of *S. pyogenes* isolates. Due to the fact that monoinfection does not always appear in the structure of pathogens of purulent-inflammatory processes in patients with acute inflammatory processes of the nasopharynx, therapy should be carried out taking into account the sensitivity of pathogens to antibiotics. On the other hand, the lack of microbiological examination of the material from patients and the results of the antibiogram may lead to complications of fungal etiology. According to the results of studies, it was found that in 6.2% of cases from the mucous membrane of the pharynx of patients with ENT pathology, bacterial-fungal associations are found. Moreover, *Candida spp.* isolated in association with bacteria showed a high level of resistance to antifungal drugs: 96.9% of the strains were resistant to nystatin, 56.3% to itraconazole, 46.9% to clotrimazole, 34.4% to ketoconazole, 21.9% fluconazole.

To understand fully the trends of the formation of antibiotic resistance, we have formed bacterial resistance profiles that display a set of inherent and acquired resistance genes of a particular clonal population. Antibiotic resistance profiles are isolate markers that can be used for epidemiological analysis of pathogens and to determination patterns of their circulation. The formed resistance profile was as follows: Z (azithromycin), C (ceftriaxone), I (ciprofloxacin), B (oxycycline), and (amoxicillin), K (clarithromycin), E (erythromycin), F (cefazolin). When interpreting the profile, a capital letter indicates the resistance of the strain to antibiotic or moderate sensitivity, □ – the sensitivity of the microorganism to the antibiotic. Our data are presented in table 3.

As we can see from the table, 24 profiles were detected among the microorganisms with multiple resistance. One hundred fifteen (23.9%) strains were resistant to 4 or more antibiotics. Among *staphylococci*, 10.3% of strains were resistant to all antibiotics, including oxacillin. Among *Klebsiella*, 29.8% of isolates were resistant to 5 antibiotics, including 3 generation cephalosporins. Five strains of streptococci were resistant to all antibiotics. The isolation of strains of microorganisms with the same antibiotic resistance profiles (strains in which the diameters of growth retardation zones around the discs with the same antibiotics are the same or differ by no more than 3 mm) from patients and from external objects of medical establishments may indicate

their hospital origin. In addition, it is believed that hospital strains of microorganisms have multiple resistance, at least up to 5 antibiotics. Marker for *staphylococci* is resistance to methicillin (oxacillin) and / or vancomycin, and for *enterobacteria* to gentamicin and / or cephalosporin antibiotics of the III-IV generations. According to our results, we can assume the circulation of hospital strains in patients undergoing outpatient treatment. Since the likelihood of infection in a hospital was minimal, we can talk about the spread of hospital isolates outside the institution. This indicates a negative trend in the spread of hospital strains that require a more careful and balanced administration of antibiotics, taking into account the antibiogram.

CONCLUSION

The microflora of the mucous membranes of the nose and throat in patients with acute inflammatory processes of the nasopharynx is represented mainly by monoisolates of *staphylococci* and *streptococci*. The sensitivity of isolated microorganisms to antibiotics varied between different types of microorganisms and between different antibiotics. Cefazolin showed efficacy against all types of microorganisms. *K. pneumoniae* strains showed the highest antibiotic resistance. Among the gram-positive *cocci*, 18 strains (4.2%) resistant to all antibiotics were isolated, which can be regarded as the circulation of hospital strains outside the medical institutions.

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ORIGINAL ARTICLE

CLINICAL AND MORPHOLOGICAL FEATURES OF THE OVARIAN BRENNER TUMOUR: CURRENT STATE OF THE PROBLEM

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ABSTRACT

Introduction: Ovarian tumours are an actual problem of present-day medicine, being one of the most difficult sections of modern oncology. The majority of ovarian tumours are of epithelial origin. The ovarian Brenner tumour represents a rare epithelial ovarian neoplasm and accounts for 1-2% of all ovarian neoplasms.

The aim of the study is to identify clinical and morphological features of ovarian Brenner tumour.

Materials and methods: The material was 5 cases of Brenner ovarian tumour, diagnosed in the study of 4 cases of operational material and 1 case of autopsy observation for the period from 2007 to 2019. Histological and immunohistochemical staining methods were used. The microspecimens were examined on an Olympus BX-41 microscope (Japan).

Results: Ovarian Brenner tumour is a rather rare pathology, the histogenesis of which is debatable. Morphological examination is the main method for its diagnosing.

Ovarian Brenner tumours developed in women of middle and old age (the average age was 51.8 years). Women with a malignant ovarian Brenner tumour were older than women with a benign variant (the average age in women with a malignant variant was 55.8 years, with a benign variant – 49.3 years). Benign ovarian Brenner tumour occurred more frequently compared with a malignant one. Malignant and benign variants of ovarian Brenner tumour were characterized by a one-sided nature of the lesion with frequent involvement in the pathological process of the left ovary. Clinically, in patients with a benign variant of the Brenner tumour in all cases an abdominal pain syndrome was determined, combined in one case with metrorrhagia. A malignant ovarian Brenner tumour was clinically manifested by severe abdominal pain syndrome, combined in one case with complaints of an increase in the size of the abdomen, and in another case with intoxication syndrome and a clinic of small bowel obstruction. In all cases a malignant ovarian Brenner tumour metastasized to the omentum and in one case also to the small intestine wall.

Macroscopically the ovarian Brenner tumour had the form of a node, the dimensions of which were significantly larger for the malignant variant compared with a benign, dense or soft consistency, on the cross section of a whitish-gray or brown color with cysts. A damaged ovary with a malignant variant of Brenner tumour significantly increased in size, while with a benign one, its size did not change or increased slightly. In all cases the malignant and benign variants of ovarian Brenner tumour were combined with various reproductive system organs pathologies (mucinous papillary cystadenoma of the ovary, serous ovarian cyst, ovarian endometriosis, endometrial hyperplasia, cervical nabothian cysts, uterine leiomyoma).

Conclusions: A study conducted by the authors revealed clinical and morphological features of a rare ovarian tumour – Brenner tumour, which will contribute to a better understanding of this pathology by the doctors of various specialties, and improve the treatment and diagnostic process.

KEY WORDS: ovarian Brenner tumour, morphology, clinical features

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INTRODUCTION

Ovarian tumours are an actual problem of present-day medicine, being one of the most difficult sections of modern oncology [1]. Ovarian tumours exhibit a wide variation in structure and behaviour. There are numerous types of ovarian

tumours, and over all they fall into benign, borderline, or atypical proliferative and malignant categories. Benign ovarian tumours may occur at any point in life but they are most common during child bearing age and constitute about 90 % of all ovarian tumours. These occur mostly in young women

between the ages of 20 to 45 years, borderline tumours occur at slightly older ages. Malignant tumours are more common in older women, between the ages of 45 and 65 years [2].

Malignant tumours of the ovary are ranked seventh among malignant diseases of the female population and eighth among the causes of women's death from oncopathology in the world. About 240,000 new cases of malignant ovarian tumours are reported annually in the world and about 150,000 women die as a result of these diseases. The highest incidence of malignant ovarian tumours has been reported in low-income and developing countries. Thus, the highest rates are in North and Central/Eastern Europe, the average are in North America, Australia and Western Europe, and the lowest – in Asia and Africa [3].

In 2018 in Ukraine, according to the National Cancer Registry, ovarian malignancies accounted for 4.9 % in the structure of the malignant neoplasms incidence of the female population, taking the 6th rank. The above pathology took 5th place in the mortality structure and 6.5 % accordingly [4].

According to the modern classification developed by experts of the World Health Organization, all ovarian tumours are divided into the following groups: epithelial tumours, mesenchymal tumours, mixed epithelial and mesenchymal tumours, sex cord-stromal tumours, mixed sex cord-stromal tumours, germ cell tumours, monodermal teratoma and somatic-type tumours arising from a dermoid cyst, germ cell-sex cord-stromal tumours, miscellaneous tumours, mesothelial tumours, soft tissue tumours, tumour-like lesion, lymphoid and myeloid tumours, secondary tumours [5].

In each case, the determination of the histological type of an ovarian tumour is based on an assessment of the tissue composition and its growth, the features of structural changes, signs of atypia, the presence and number of mitoses, as well as the characteristics of the cellular composition [6]. Unusual mosaic structure of the microscopic structure of ovarian neoplasms, diversity of their combinations, fuzzy boundaries between tumours of different degrees of maturity, abundance of metastatic lesions complicate the diagnostic process and, at times, create obstacles to the rational planning of therapeutic measures (the volume of surgical intervention and nature of conservative therapy), determining the prognosis of the course of the disease [1, 6].

The majority of ovarian tumours are of epithelial origin [7]. The ovarian Brenner tumour represents a rare epithelial ovarian neoplasm and accounts for 1-2% of all ovarian neoplasms [8]. Brenner tumours very rarely can occur in other locations, including the testis [9]. Identified in 1907 by a German pathologist Fritz Brenner, ovarian Brenner tumours are now subclassified into benign, borderline (atypical proliferative) and malignant categories [8]. The benign ones are the most frequent, representing about 95%, the borderline represent about 4-5%, and the malignant ones less than 1% [10]. Malignant Brenner tumour was firstly reported by von Numbers in 1945 [8]. In 1971 Roth and Sternberg reported borderline type of the ovarian Brenner tumour [11].

The issue of histogenesis of Brenner ovarian tumour is currently debatable. Historically proposed origins have included the ovarian surface epithelium, mesonephric remnants, rete

ovarii, mucinous tumours, teratomas [12]. The most widely accepted hypothesis is that the tumour develops from Walthard cell nests, which are metaplastic transitional epithelium usually seen in the paratubal region [11]. It was noted that the transitional-like epithelium of Walthard cell nests is morphologically identical to the epithelial nests in Brenner tumours [12].

Given the rarity of the ovarian Brenner tumour, it seems relevant to analyze our own cases from practice in order to study its clinical and morphological features, which will contribute to a better understanding of this pathology and improve the diagnostic and treatment process.

THE AIM

The aim of the study is to identify clinical and morphological features of ovarian Brenner tumour.

MATERIALS AND METHODS

The material was 5 cases of Brenner ovarian tumour, diagnosed, taking into account modern morphological criteria [5], in the study of 4 cases of operational material and 1 case of autopsy observation for the period from 2007 to 2019.

The material was fixed in 10% neutral formalin. Tissue consolidation fixed in formalin was reached through alcohol of increased concentration, the Nikiforov liquid (96% alcohol and diethyl ester in a 1:1 ratio), chloroform and filled in wax. Serial sections $4-5 \times 10^{-6}$ m thick were made from blocks for hematoxylin and eosin staining. In one case an immunohistochemical study was performed with monoclonal antibodies to cytokeratin 7, 20 and vimentin. The microspecimens were examined on an Olympus BX-41 microscope (Japan).

RESULTS AND DISCUSSION

Diagnosis of a Brenner tumour using existing imaging methods is extremely difficult, and sometimes impossible. Considering the fact that the Brenner tumour of the ovary does not have specific signs, the results of ultrasound examination (fig. 1), computed tomography and magnetic resonance imaging only suggest the presence of the above tumour process in the patient. Pelvic organs are examined with a vaginal probe with frequency of 7-12 MHz, which allows you to visualize the structure of the uterus, endometrium and ovaries with high resolution.

For large tumours transabdominal scanning using a convex probe with a frequency of 3-5 MHz is used. A tumour is more often of a solid structure, with fairly clear, slightly tuberous contours, sometimes a somewhat heterogeneous structure. Internal anechogenic inclusions corresponding to degenerative changes are often determined. A «stony» echostructure with the effect of lateral attenuation may occur. Sometimes a liquid component and calcifications may be present. The use of color Doppler mapping reveals single blood flow loci in the tumour structure. Elastography is not specific.

The main role in the diagnosis of Brenner ovarian tumour is assigned to morphological research methods. Microscopic examination of our own material in three cases revealed a

benign variant of ovarian Brenner tumour. The latter was constructed from connective tissue, between the fibers of which it was determined the islets of various shapes and sizes, formed by polyhedral cells of epithelial nature, which often resembled a transitional epithelium of the urinary tract (fig. 2a, 2b). Some scientists note that the epithelial cells have a characteristic coffee bean nucleus with longitudinal grooves and pale cytoplasm [13]. In some places in the center of the islets we revealed round or round-oval cavities with pink homogeneous contents and desquamated cells (fig. 2b). In some fields of view between the connective tissue fibers, areas of dystrophic calcification and hyalinosis were noted.

Benign Brenner tumours are mainly asymptomatic and may have incidental findings particularly if small. In those symptomatic patients, the most common symptoms include abnormal vaginal bleeding, perceptible lumps and pain associated or not with the pelvic masses [14]. Infrequently, this neoplasm may manifest by Meigs syndrome, which includes a benign ovarian tumour, accompanied by ascites, hydrothorax and less often hydropericardium [15].

In our study a benign Brenner tumour was not diagnosed in three cases by clinicians and in the clinical diagnosis was documented as an ovarian cyst. Given the presence of multiple uterine leiomyoma in all of the above cases, these women underwent uterine extirpation (in one case with the cervix) with both appendages. This pathology was clinically manifested in all three cases by abdominal pain syndrome, which in one case was combined with bleeding from the uterine cavity.

The morphological study revealed a combination of a benign Brenner tumour with other pathologies of the ovary, uterus and cervix. Thus, the above pathology in one case combined with mucinous papillary cystadenoma of the ovary (fig. 3), endometriosis of the ovary (fig. 4), glandular-cystic endometrial hyperplasia, cervical nabothian cysts, in three cases with multiple uterine leiomyoma.

Other scientists noted also the combination of a benign Brenner tumour with various pathologies of the reproductive system organs. Brenner tumours coexist with other tumoural lesions, such as cystic mucinous tumour, serous cyst adenoma or the cystic dermoid teratoma in 25-36 % of the cases. The presence of other tumoural or non-tumoural lesions in the female genital system would be due to the fact the Brenner tumours contain cells with a secretory function producing estrogens, hormones that are responsible for the emergence of various lesions, from endometrial hyperplasia with vaginal bleeding to ovarian tumours [10]. Bleeding from the uterine cavity and morphologically diagnosed glandular-cystic endometrial hyperplasia noted by us can also possibly be a manifestation of the estrogenic effect of the Brenner tumour.

E. Kuhn et al. showed that luteinized stromal cells immediately adjacent to the epithelial nests of the Brenner tumours, as well as the epithelial nests themselves, are involved in androgen biosynthesis in view of the presence of AKR1C3, a key enzyme in the production of androgens, in these cells and that hormone stimulation therefore probably has a role in the development of Brenner tumours as the epithelial component contains androgen receptor [16]. The androgen-producing activity of a Brenner tumour may cause masculinization in patients [17].

In our study, in two cases, a Brenner malignant tumour was diagnosed morphologically, not detected or correctly interpreted by the attending physicians in patients.

In the first case, a woman had clinically determined abdominal pain, complaints of an increase in the size of the abdomen, a large ovarian cyst and multiple uterine leiomyoma were documented in the clinical diagnosis. This woman underwent surgery, during which the uterus with the cervix and both appendages, a fragment of the omentum were removed. The results of a morphological study of the surgical material revealed a combination of a malignant ovarian Brenner tumour with a serous ovarian cyst, uterine leiomyoma, cervical nabothian cysts. Morphologically, the tumour process was characterized by the presence of islets of squamous and transitional cancer separated by connective tissue layers. In some fields of view in the epithelial nests and islets we noted the structure of adeno-squamous cell carcinoma with the signs of mucus formation and the presence of signet-ring cells (fig. 5a, 5b). In the obtained omentum fragment, metastases of the ovarian Brenner malignant tumour were noted.

In the second case, a woman had a pronounced pain syndrome, signs of intoxication and small bowel obstruction. Cancer of the small intestine was documented in the clinical diagnosis. The woman died. An autopsy and the results of a histological examination of autopsy material revealed a malignant ovarian Brenner tumour with metastases in the omentum, small intestine (fig. 6), which has led to the development of intestinal obstruction, serous-fibrinous peritonitis and intoxication. The cause of death was the intoxication of combined genesis due to the presence of a malignant ovarian Brenner tumour and the development of small bowel obstruction with serous-fibrinous peritonitis. In this case a malignant ovarian Brenner tumour was combined with uterine leiomyoma, glandular-cystic endometrial hyperplasia. It was morphologically characterized by the presence of invasive nests and foci of neoplastic cells with pronounced proliferation and atypia, the structure of which in most fields of view corresponded to transitional cell carcinoma (fig. 7) and in some fields of view – squamous cell carcinoma. In some fields of view in the center of the nests were found the cavities filled with eosinophilic masses with desquamated cells.

Benign ovarian Brenner tumour, as shown by the analysis of our cases, has a fairly clear morphological picture. However, the nosological specification of a malignant ovarian Brenner tumour sometimes causes certain difficulties. A Brenner tumour is known to undergo both monomorphic and dimorphic differentiation during malignancy. The latter pathway leads to the formation of adeno-squamous cell carcinoma with the signs of intracellular mucus formation in the glandular component. Diagnosis of this variant of the Brenner tumour is not difficult. However, in cases of unilaterally developed monomorphic variants of this nosology, the complexity can be insurmountable. In these patients, the tumour has the structure of either purely squamous or transitional cell carcinoma. Then it is required to find the structures of the previous or coexisting benign Brenner tumour and establish transitions between the two processes [1]. In the studied cases of malignant ovarian Brenner tumours we revealed coexisting structures of a benign Brenner tumour.

Despite the fact that microscopic examination remains the first method in the diagnosis of ovarian Brenner tumours, in



Fig. 1. Ultrasound investigation. Transabdominal scan. 3-5 MHz multi-frequency convex sensor. A solid hypoechoic tumour is visualized in the right ovary of a somewhat heterogeneous echostructure with fairly clear tuberos contours, 8×9 cm in size.

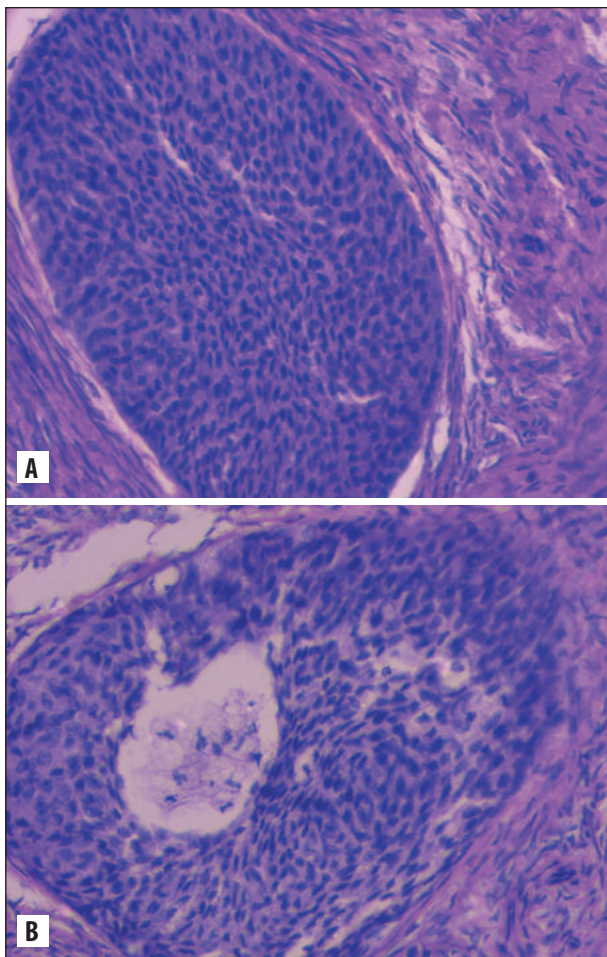


Fig. 2. There are the islets of epithelial cells resembling transitional epithelium (a, b) in the benign ovarian Brenner tumour. Some islands are characterized by the presence of round-oval cavity filled with eosinophilic masses and desquamated cells (b). Stained with hematoxylin and eosin, × 100.

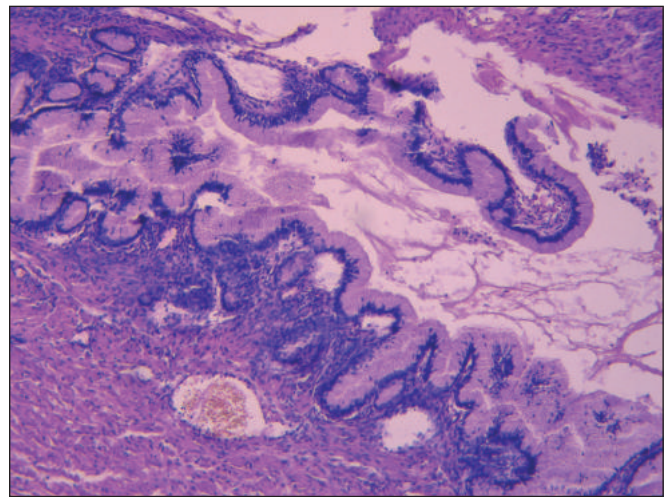


Fig. 3. Mucinous papillary cystadenoma of the ovary. Stained with hematoxylin and eosin, × 100.

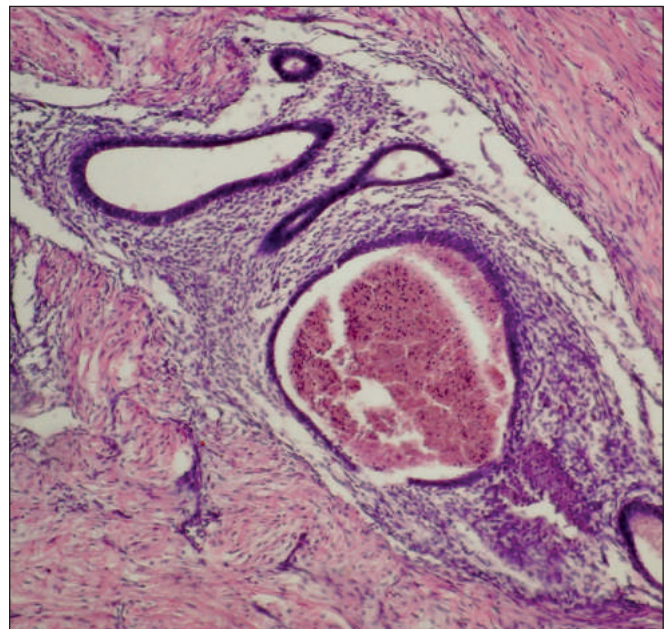


Fig. 4. Ovarian endometriosis. Stained with hematoxylin and eosin, × 100.

some cases an immunohistochemical study is necessary to determine the exact histotype of the tumour [17]. Brenner tumours, including benign, borderline and malignant, express cytokeratin 7, p63, S100P, GATA3, uroplakin and thrombomodulin, but do not express, or only focally express, cytokeratin 20 [5, 18]. The fibrous stroma of a Brenner tumour expresses vimentin [19].

In one case of a malignant ovarian Brenner tumour, a brief analysis of which was published earlier [20], during the morphological diagnosis using routine staining methods, difficulties arose in establishing the histogenetic origin of the tumour, which required an immunohistochemical study. Immunohistochemically it was detected the expression of cytokeratin 7 by the epithelial tumour cells (fig. 8), vimentin by the stromal cells (fig. 9), and a negative reaction with monoclonal antibody to cytokeratin 20.

Molecular investigations have been carried out to explain the progression of the benign ovarian Brenner tumour to the atypical proliferative Brenner tumour, as well as the progression

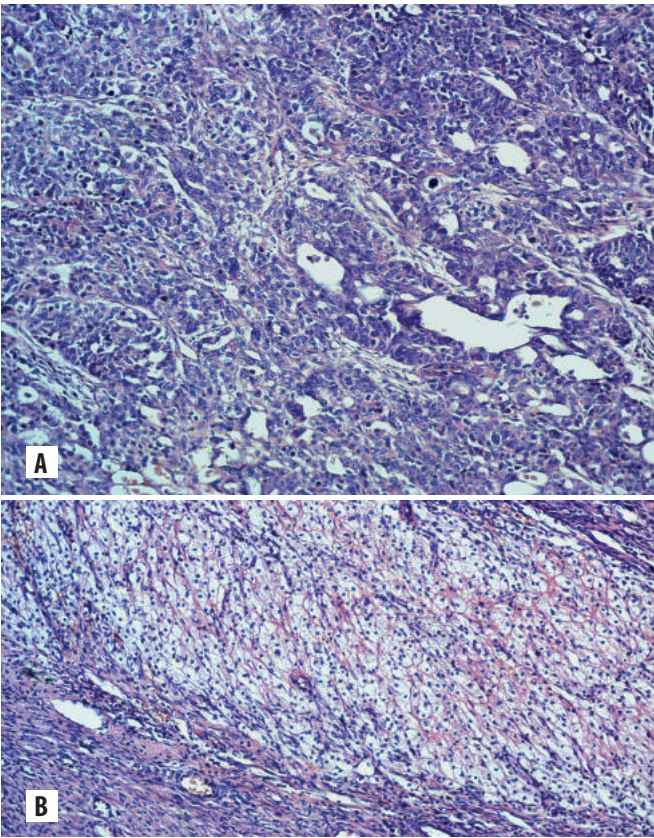


Fig. 5. In the epithelial nests and islets, the structure of adeno-squamous cell carcinoma (a) with the signs of mucus formation and the presence of signet-ring cells (b) is noted. Stained with hematoxylin and eosin, $\times 100$.

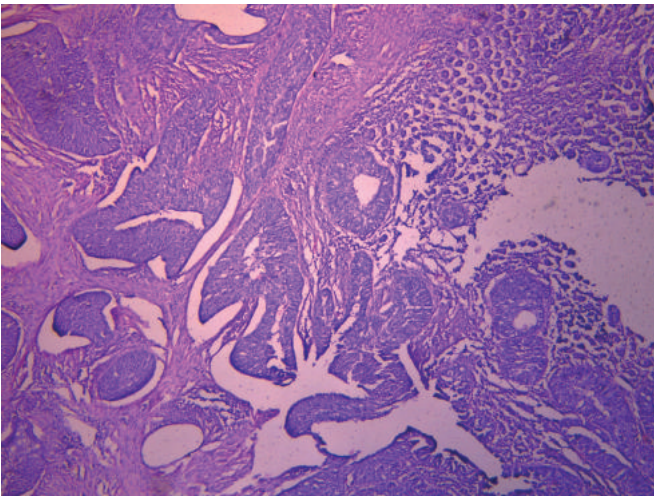


Fig. 6. Metastasis of a malignant ovarian Brenner tumour in the small intestine wall. Stained with hematoxylin and eosin, $\times 40$.

of the benign and atypical proliferative Brenner tumour to the malignant one [16].

A comparative analysis of the material showed that the connective tissue component was more pronounced in the benign Brenner tumour than the malignant variant, which was also noted by other scientists [17].

Malignant ovarian Brenner tumour compared with a benign variant is more often manifested by any symptoms. The clinical manifestations of malignant Brenner tumour are various. They in-

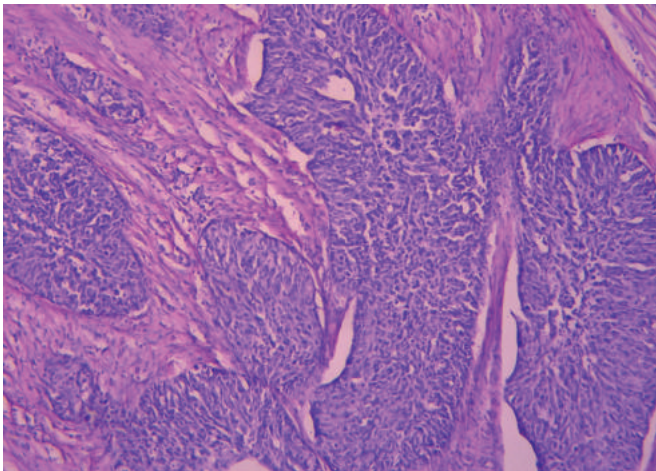


Fig. 7. Among connective tissue fibers, there are the invasive nests and foci of neoplastic cells with pronounced proliferation and atypia, the structure of which resembles transitional cell carcinoma. Stained with hematoxylin and eosin, $\times 100$.

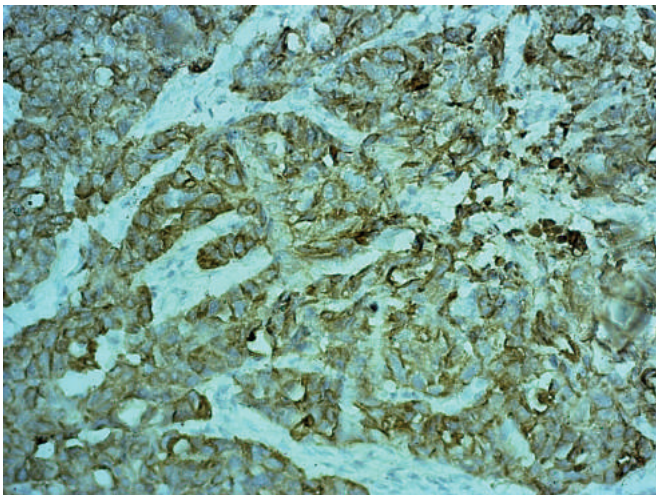


Fig. 8. Expression of cytokeratin 7 by the epithelial cells of a malignant ovarian Brenner tumour. Immunohistochemical reaction with monoclonal antibody to cytokeratin 7, $\times 200$.

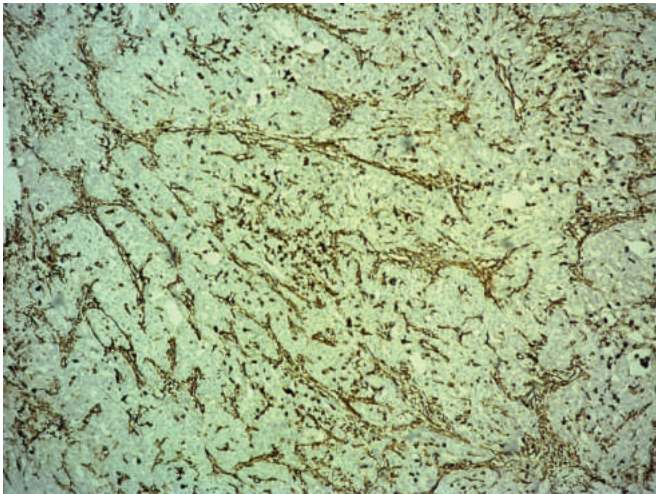


Fig. 9. Expression of vimentin by the connective tissue stroma of a malignant ovarian Brenner tumour. Immunohistochemical reaction with monoclonal antibody to vimentin, $\times 200$.

clude abdominal pain, metrorrhagia, nausea, vomiting, back pain. Tumour-induced cachexia and weight loss can also be seen [11].

According to the literature data, malignant Brenner tumours metastasize so rare [21]. It was described that the malignant Brenner tumour can metastasize to the skin, lungs, central nervous system or shows tendency for intraperitoneal dissemination [15]. In our study in all cases a malignant ovarian Brenner tumour metastasized to the omentum, and in one case also to the small intestine wall.

In a macroscopic examination, in the case of a diagnosed benign variant, the tumour resembled a node with a diameter of 0.3 to 0.7 cm with dense consistency, round or round-oval in shape, in some places with small cysts with a diameter of 0.1 to 0.3 cm with mucus-like translucent contents. The size of the ovary in a benign Brenner tumour did not change or increased slightly. Macroscopically, a malignant Brenner tumour resulted in a pronounced ovarian enlargement in size and was represented by a node from 1.0 to 12.0 cm in diameter, whitish-gray or brown colour, dense or soft consistency, with cysts from 0.4 cm to 1.0 cm in diameter with whitish translucent or brown contents.

Significantly large sizes of the Brenner tumour in malignant variant compared with the benign variant were also identified by other scientists. Accordingly, it was noted that the size of Brenner tumours correlated with histologic grade: most benign tumours were, in general, 5-6 cm in diameter, occasionally exceeding 10 cm. Borderline and malignant Brenner tumours can reach 30 cm [13].

A Brenner tumour, according to the literature, can have both unilateral and bilateral nature of the lesion. A more frequent involvement in the pathological process of the left ovary compared with the right was also found. Benign Brenner tumour, as a rule, has a one-sided nature of the lesion [22]. In malignant Brenner tumours bilateral lesions were found in 5-14 % of cases, in other cases it was unilateral lesions [23]. In our study, in all cases, Brenner tumour had a one-sided nature of the lesion. Its benign variant in all cases was detected in the left ovary and malignant – in the first case in the right ovary and in the second case in the left ovary.

The average age of patients with Brenner ovarian tumour was 51.8 years. With its malignant variant, the average age (55.8 years) of patients was higher compared with the average age (49.3 years) of patients with a benign variant. It has been noted by the scientists that Brenner tumours develop in premenopause or menopause women. Numerous studies have showed that the average age at which these tumours are diagnosed is 46-63 years. Benign Brenner tumours seem to develop also in women aged between 30 and 59 years, while malignant and borderline tumours develop in older women (45-60 years) [10].

In this study, we did not find cases of a borderline variant of Brenner tumour. Borderline Brenner tumours compared to benign variant showed several large, irregular-shaped nests of tumour cells, most of which displayed cystic dilatation with intraluminal papillary or polypoid protrusions [24]. Borderline Brenner tumours have areas of atypia coexistent with benign components.

The cytologic findings that qualify a Brenner tumour as proliferative or borderline are as follows: mucinous or squa-

mous metaplasia in transitional epithelium, complex glandular formation, nuclear atypia including hyperchromatic nuclei, coarse chromatin clumping, prominent nucleoli and increased mitotic activity. Focal areas resembling noninvasive papillary transitional carcinoma or papillary squamous carcinoma in situ have also been reported in borderline Brenner tumours. Some borderline tumours contain localized calcification [11].

Due to the rarity of ovarian Brenner tumor treatment strategy of patients with this pathology is not entirely clear. The main method is surgery. Issues related to radiation therapy and chemotherapy are still debatable and require further research. The prognosis of benign ovarian Brenner tumours is favorable. Borderline tumours do not metastasize, but can relapse. The prognosis of malignant ovarian Brenner tumours depends on the presence or absence of metastases and the prevalence of the tumour process [5, 17].

CONCLUSIONS

The analysis of own cases from practice of ovarian Brenner tumour has shown the following:

1. Ovarian Brenner tumour is a rather rare pathology, the histogenesis of which is debatable. Morphological examination is the main method for its diagnosing.
2. Ovarian Brenner tumours developed in women of middle and old age (the average age was 51.8 years). Women with a malignant ovarian Brenner tumour were older than women with a benign variant (the average age in women with a malignant variant was 55.8 years, with a benign variant – 49.3 years). Benign ovarian Brenner tumour occurred more frequently compared with a malignant one. Malignant and benign variants of ovarian Brenner tumour were characterized by a one-sided nature of the lesion with frequent involvement in the pathological process of the left ovary. Clinically, in patients with a benign variant of the Brenner tumour in all cases an abdominal pain syndrome was determined, combined in one case with metrorrhagia. A malignant ovarian Brenner tumour was clinically manifested by severe abdominal pain syndrome, combined in one case with complaints of an increase in the size of the abdomen, and in another case with intoxication syndrome and a clinic of small bowel obstruction. In all cases a malignant ovarian Brenner tumour metastasized to the omentum and in one case also to the small intestine wall.
3. Macroscopically the ovarian Brenner tumour had the form of a node, the dimensions of which were significantly larger for the malignant variant compared with a benign, dense or soft consistency, on the cross section of a whitish-gray or brown color with cysts. A damaged ovary with a malignant variant of a Brenner tumour significantly increased in size, while with a benign one, its size did not change or increased slightly. In all cases the malignant and benign variants of ovarian Brenner tumour were combined with various reproductive system organs pathologies (mucinous papillary cystadenoma of the ovary, serous ovarian cyst, ovarian endometriosis, endometrial hyperplasia, cervical nabothian cysts, uterine leiomyoma).

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

WPŁYW KORZYSTANIA Z SYSTEMU NIGHTSCOUT NA WYRÓWNANIE METABOLICZNE CUKRZYCY, BEZPIECZEŃSTWO UŻYTKOWANIA ORAZ OBECNOŚĆ POWIKŁAŃ

EFFECT OF USING THE NIGHTSCOUT SYSTEM ON METABOLIC CONTROL, SAFETY, AND INCIDENCE OF COMPLICATIONS IN PATIENTS WITH DIABETES MELLITUS

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Oskar Kublin, Mariusz Stępień

ZAKŁAD PROPEDEUTYKI INTERNY I FARMAKOLOGII SPOŁECZNEJ, KATEDRA NEFROLOGII I NADCIŚNIENIA TĘTNICZEGO, USK IM. WAM – CENTRALNY SZPITAL WETERANÓW, ŁÓDŹ, POLSKA

STRESZCZENIE

Wstęp: System Nightscout jest darmowym rozwiązaniem DIY – Do It Yourself. W Polsce system ten pojawił się w 2016 roku. Koncepcją projektu jest umożliwienie wglądu w pomiary z systemu ciągłego monitorowania stężenia glukozy w płynie śródtkankowym przez osoby uprawnione.

Cel pracy: Ocena funkcjonalności systemu Nightscout oraz jego wpływu na wyrównanie metaboliczne cukrzycy, bezpieczeństwo użytkowania oraz obecność powikłań u pacjentów z cukrzycą typu 1.

Materiały i metody: W badaniu wzięło udział 98 osób chorych na cukrzycę typu 1. Badanie zostało przeprowadzone przy pomocy anonimowej ankiety internetowej, wśród członków grupy „Nightscout Polska (Poland)” na portalu społecznościowym Facebook.

Wyniki: Liczba epizodów ciężkich hipoglikemii z utratą przytomności i koniecznością podania dodatkowych leków była wyższa przed rozpoczęciem korzystania z systemu Nightscout – wystąpiła u 3 dorosłych i wynosiła od 1 do 2 epizodów. Podczas korzystania z systemu Nightscout nie odnotowano incydentów ciężkich hipoglikemii. W grupie dzieci korzystających z systemu Nightscout odnotowano redukcję liczby epizodów kwasicy ketonowej z 5 incydentów przed rozpoczęciem korzystania do 2 epizodów podczas korzystania z Nightscout. U dorosłych użytkowników systemu Nightscout nastąpiła redukcja z 3 epizodów do 0. Wartości hemoglobiny glikowanej osób korzystających z systemu Nightscout były niższe niż ich wartości przed rozpoczęciem korzystania z tego systemu. Wykazano również, że osoby korzystające z systemu Nightscout miały niższą hemoglobinę glikowaną niż osoby z grupy kontrolnej.

Wnioski: Korzystanie z systemu Nightscout może pozytywnie wpływać na bezpieczeństwo stosowanej insulinoterapii oraz proces leczenia cukrzycy typu 1. System Nightscout może być udoskonaleniem systemu systemu ciągłego monitorowania śródtkankowego stężenia glukozy.

SŁOWA KLUCZOWE: cukrzyca typu 1, CGM – continuous glucose monitoring, DIY – do it yourself, Nightscout

ABSTRACT

Introduction: The Nightscout system is a free Do It Yourself solution. This system appeared in Poland in 2016. The concept of the project is to provide insight into measurements from the system of continuous glucose monitoring in interstitial fluid by authorized persons.

The aim: The study was carried out to assess the functionality of the Nightscout system and its effect on metabolic control, safety and the incidence of complications in patients with type 1 diabetes mellitus.

Material and methods: The study comprised 98 patients with type 1 diabetes. The study was conducted online using an anonymous questionnaire targeted at members of the “Nightscout Polska (Poland)” group on the Facebook.

Results: Severe hypoglycaemic episodes with loss of consciousness were more frequent before using the Nightscout system and were reported by 3 adults (1-2 episodes). No severe hypoglycaemic episodes were reported when using the Nightscout system. The number of ketoacidosis episodes was reduced from 5 before using the Nightscout system to 2 episodes during the use of the system in children, and from 3 to 0 episodes in adults. Levels of glycated haemoglobin were lower in patients using the Nightscout system, both compared to control groups and values before the use it. In people using the Nightscout system glycated hemoglobin values were lower than their values before using this system. It was also shown that people using the Nightscout system had lower glycated hemoglobin than people from the control group.

Conclusions: Using the Nightscout system can positively affect the safety of insulin therapy and the treatment process of type 1 diabetes. The Nightscout system can be an improvement of the system of continuous glucose monitoring in interstitial fluid.

KEY WORDS: diabetes mellitus type 1, CGM – continuous glucose monitoring, DIY – do it yourself, Nightscout

WSTĘP

W celu prawidłowego leczenia pacjenci chorujący na cukrzycę zobligowani są do częstych kontroli wartości glikemii. Obecnie coraz częściej są w tym celu wykorzystywane systemy ciągłego monitorowania stężenia glukozy w płynie śródtkankowym. W sprzedaży dostępnych jest kilka systemów CGM – *continous glucose monitoring* oraz system FGM – *flash glucose monitoring* [1–4]. Pierwszy z nich, czyli system CGM podaje wartość glukozy co 5 minut. Natomiast drugie rozwiązanie – FGM, wymaga od użytkownika zbliżenia urządzenia w celu dokonania pomiaru. Osoby korzystające z wymienionych rozwiązań uzyskują łatwiejszy wgląd do aktualnych wartości stężenia glukozy, ponadto wykonanie pomiaru jest bezbolesne. Wspomniane systemy zostały poddane badaniom klinicznym, w których potwierdzono ich pozytywny wpływ na terapię cukrzycy. Pacjenci korzystający z systemów śródtkankowego pomiaru stężenia glukozy prezentowali niższe wartości hemoglobiny glikowanej, nastąpiło u nich zmniejszenie czasu trwania i liczby epizodów hipoglikemii oraz zwiększył się czas przebywania w zakresie prawidłowych wartości stężenia glukozy [5–7]. Pomimo ułatwień jakie dają systemy śródtkankowego pomiaru stężenia glukozy, pacjenci nadal obarczeni są koniecznością podejmowania wielu decyzji terapeutycznych. W przypadku dzieci w podejmowaniu tych decyzji pomagają dorośli, jednak staje się to problematyczne na odległość, np. gdy dziecko jest w szkole. W 2014 roku rodzice dzieci chorujących na cukrzycę typu 1 stworzyli projekt, który miał za zadanie ułatwić im kontrolę nad swoimi podopiecznymi [8].

System Nightscout jest darmowym rozwiązaniem DIY – *Do It Yourself*. W Polsce system ten pojawił się w 2016 roku [9]. Koncepcją projektu jest umożliwienie wglądu w pomiary z systemu ciągłego monitorowania stężenia glukozy w płynie śródtkankowym przez osoby uprawnione. Przesył danych odbywa się za pośrednictwem internetu, a dane przechowywane są na zewnętrznych serwerach. Wgląd do pomiarów może zostać udostępniony na różnych urządzeniach z dostępem do internetu (telefon, komputer, zegarek).

Osoba chorująca na cukrzycę musi być zaopatrzona w urządzenie do monitorowania stężenia glukozy w płynie śródtkankowym. Nightscout współpracuje zarówno z systemami CGM, jak i FGM, jednak w przypadku tego drugiego niezbędna jest akcesoryjna nakładka odczytująca dane z sensora Libre poprzez NFC – *near field communication* oraz przesyłająca je dalej poprzez Bluetooth [10–12]. Spośród systemów CGM dostępnych w Polsce system Nightscout współpracuje z urządzeniem firmy Roche (Eversense XL), urządzeniami firmy Dexcom (G5, G4 Platinum) oraz urządzeniami firmy Medtronic (Guardian Connect, MiniMed 640G, serie 7XX i 5XX oraz VEO). W zależności od rodzaju posiadanego systemu CGM odmienny jest sposób komunikacji. Do urządzeń firmy Medtronic serii 5XX, 7XX, VEO oraz 640G podłączany jest dodatkowy moduł komunikacyjny za pomocą przewodu USB OTG. System Dexcom G4 Platinum można podłączyć kablem USB OTG lub bezprzewodowo przy wykorzystaniu dodat-

kowego urządzenia xBridge (komunikacja Bluetooth) lub Parakeet (komunikacja GSM). System Guardian Connect wykorzystuje firmowe konto w serwisie CareLink, które trzeba zintegrować ze stroną Nightscout.

CEL PRACY

Dotychczas nie przeprowadzono oceny klinicznej przydatności systemu Nightscout u polskich pacjentów z cukrzycą typu 1. Biorąc to pod uwagę, niniejsze badanie przeprowadzono w celu oceny funkcjonalności tego systemu oraz jego wpływu na wyrównanie metaboliczne cukrzycy, bezpieczeństwo użytkowania oraz obecność powikłań u wyżej wymienionych chorych.

MATERIAŁY I METODY

Badanie zostało przeprowadzone przy pomocy anonimowej ankiety internetowej, która została wysłana do członków grupy Nightscout Polska (Poland) na portalu społecznościowym Facebook. Dodatkowo informacja o przeprowadzanym badaniu została ogłoszona przez administratora wewnątrz grupy [13]. Członkowie grupy po kontakcie z badaczem otrzymywali informację dotyczącą celu badania i jego metodyki, link do ankiety oraz podstawowe wskazówki techniczne. Wszystkie osoby uczestniczące w badaniu wyraziły dobrowolną zgodę oraz miały możliwość zadawania pytań. Grupa ta została wybrana, ponieważ w jej skład wchodziły użytkownicy systemu Nightscout, krajowi pionierzy tego rozwiązania oraz osoby zainteresowane tym projektem. W ankiecie oprócz pytań ogólnych (płeć, rok urodzenia, typ cukrzycy i czas jej trwania) zapytano dodatkowo o czas korzystania z systemu Nightscout, jak również poproszono uczestników badania o podanie informacji dotyczących stopnia wyrównania metabolicznego cukrzycy, tj. wartości hemoglobiny glikowanej (HbA1C), rodzaju stosowanej terapii, obecność wczesnych i późnych powikłań cukrzycy oraz częstości występowania epizodów hipoglikemii (przed korzystaniem z systemu Nightscout i podczas niego). Dodatkowo badanych poproszono o podanie wad i zalet użytkowania systemu Nightscout oraz rodzaju urządzeń, na których wyświetlane są przez nich wyniki. Ze względu na poruszany w grupie temat automatycznej modyfikacji podawanej dawki insuliny w układzie „zamkniętej pętli” w ankiecie uwzględniono również jedno pytanie odnoszące się do tego zagadnienia. W przypadku wypełniania ankiety przez opiekunów diabetyków zaznaczono, że pytania w ankiecie odnoszą się do osoby chorej. Respondenci mogli wypełniać ankietę przez 4 tygodnie na przełomie stycznia i lutego 2019 roku. Ankietowani wyrazili dobrowolną zgodę na wypełnienie ankiety oraz wykorzystanie odpowiedzi w sposób anonimowy do celów naukowych.

Badanie zostało zatwierdzone przez Komisję Bioetyczną przy Uniwersytecie Medycznym w Łodzi.

ANALIZA DANYCH

Analizę statystyczną przeprowadzono przy użyciu pakietu IBM SPSS Statistics w wersji 25. W celu porównania częstości

Tabela I. Charakterystyka kliniczna badanych grup

Dane kliniczne	Rodzaj grupy	Dorośli		Dzieci	
		A	KA	C	CK
Liczebność grupy		20	9	49	10
Wiek (lata)		39,95 ± 6,08	39,55 ± 5,65	8,8 ± 3,23	9,4 ± 3,23
Płeć M/K (n/%)		5 / 15 25%/75%	3 / 6 33,3%/66,6%	22 / 27 44,9%/55,1%	5 / 5 50%/50%
Czas trwania cukrzycy (lata)		8,52 ± 9,08	17 ± 16,05	3,94 ± 1,86	5,5 ± 2,55
Czas użytkowania systemu Nightscout (miesiące)		16,35 ± 7,65		17,22 ± 13,74	
Rodzaj insulinoterapii (OPI ^a /WWI ^b)		18 / 2	7 / 2	48 / 1	10 / 0
Retinopatia		3	3	0	0
Nadciśnienie tętnicze		0	1	0	0
Neuropatia		0	1	0	0

a – osobista pompa insulinowa

b – wielokrotne wstrzyknięcia insuliny

występowania odpowiedzi w ramach danego pytania zastosowano test chi-kwadrat. Do porównania wyników dwóch niezależnych grup (osób stosujących system Nightscout i grupa kontrolna, dorośli i dzieci) wykorzystano test U Manna Whitney'a, gdy dane miały charakter ilościowy, a w przypadku danych: jakościowych, nominalnych, kategoryalnych użyto test chi-kwadrat niezależności bądź test dokładny Fishera, gdy liczebność oczekiwana była mniejsza niż 5.

W celu określenia skuteczności stosowania systemu Nightscout przeprowadzono analizę za pomocą testu znaków rangowanych Wilcozona, gdy dane były ilościowe bądź testu McNemara, gdy dane były nominalne. Jako poziom istotności na potrzeby interpretacji analiz, przyjęto $\alpha = 0,05$. Wyniki przedstawiono jako średnie ± odchylenia standardowe (SD).

WYNIKI

Ankiety wypełniło 98 osób. Wszyscy uczestnicy badania chorowali na cukrzycę typu 1. Średni czas wypełniania formularza wynosił około 5 minut. Do grupy badanej zaliczone zostały osoby, które w wypełnionej ankiecie zadeklarowały korzystanie z systemu Nightscout. Ze względu na obowiązkowość korzystania z systemów CGM lub FGM (w połączeniu z dodatkową nakładką) w celu użytkowania systemu Nightscout, z grupy kontrolnej wykluczono 3 osoby korzystające wyłącznie z tradycyjnych glukometrów oraz 7 osób, u których sposób kontroli glikemii nie był znany. Wśród wszystkich uczestników badania zakwalifikowanych do dalszej analizy kobiety stanowiły 60,2% (n=53) respondentów, a mężczyźni 39,8% (n=35). Średni wiek wszystkich uczestników wynosił 19,10 ± 15,21 roku, z czego 59 osób (67%) liczyło mniej niż 18 lat. Zdecydowana większość uczestników badania (n=69, tj. 78,4%) zadeklarowała korzystanie z systemu Nightscout. Wśród badanych najdłuższy czas posługiwania się tym systemem wynosił 50 miesięcy, zaś najkrótszy ograniczony był do miesiąca. Osoby korzystających z systemu Nightscout podzielono na 2 podgrupy: A – dorosłych (n=20) oraz C – dzieci (n=49) (Tab. I). Grupa

kontrolna (K) obejmująca osoby niekorzystające z systemu Nightscout liczyła 19 osób (21,6%), z tego pacjenci dorośli liczyli 9 osób (podgrupa KA), a dzieci (podgrupa KC) 10 osób. W grupie dzieci znajdowało się 27 chłopców i 32 dziewczynki w wieku od 1 roku do 17 lat, średni wiek w grupie C wyniósł 8,8 ± 3,23 roku, a w grupie kontrolnej KC był on nieco wyższy i wyniósł 9,4 ± 3,23 (Tab. I). W grupie osób dorosłych znajdowało się łącznie 8 mężczyzn i 21 kobiet w wieku od 31 do 55 lat, średni wiek w grupie osób korzystających z systemu Nightscout A wyniósł 39,95 ± 6,08, a w grupie kontrolnej dorosłych KA było to odpowiednio 39,55 ± 5,65 roku (Tab. I).

Średni czas od rozpoznania cukrzycy był większy u dorosłych i wynosił odpowiednio: 8,52 ± 9,08 dla grupy A oraz 17 ± 16,05 dla grupy KA, natomiast wśród dzieci było to 3,94 ± 1,86 dla grupy C oraz 5,5 ± 2,55 dla grupy KC (Tab. I). Wśród wszystkich respondentów przeważająca większość (n=83, tj. 94,3%) była leczona za pomocą osobistej pompy insulinowej, pozostałe osoby (n=5, tj. 5,7%) stosowały insulinoterapię metodą wielokrotnych wstrzyknięć insuliny (Tab. I). Wszystkie osoby wchodzące zarówno w skład grup badanych, jak i kontrolnych korzystały z systemów śródtkankowego pomiaru stężenia glukozy, zarówno z FGM (*Flash Glucose Monitoring*) – *Freestyle Libre*, jak i CGM (*Continuous Glucose Monitoring*) – sensory Dexcom, Enlite oraz Eversense.

U 3 osób (3,4%) z grupy A oraz również u 3 (33,3%) z grupy KA stwierdzono retinopatię cukrzycową (Tab. I). W grupie KA u 1 pacjenta (11,1%) stwierdzono neuropatię, w tej grupie również u 1 chorego (11,1%) rozpoznano nadciśnienie tętnicze. U 1 pacjenta (5%) w grupie A wystąpiły zaburzenia lękowe. W odpowiedziach na pytania o powikłania badani zgłaszali ponadto występowanie chorób przewlekłych, które nie były związane z cukrzycą.

WYRÓWNANIE METABOLICZNE

Na pytanie o wartość HbA1C przed rozpoczęciem korzystania z systemu Nightscout w grupie A 5 osób nie udzie-

liło odpowiedzi, gdyż nie pamiętało wartości, jakkolwiek deklarowali, iż była ona wyższa niż w trakcie korzystania z systemu Nightscout. Natomiast w grupie C 21 ankietowanych wybrało odpowiedź porównawczą opartą na ich subiektywnej ocenie i zadeklarowało, że wcześniej była ona wyższa, dla 1 uczestnika wynik był porównywalny, a tylko 1 osoba podała, że była niższa. Natomiast średnie wartości HbA1C w trakcie stosowania tego systemu wynosiły odpowiednio w grupie A $6,63 \pm 0,61\%$, a w grupie C $6,67 \pm 0,76\%$ (Tab. II). W grupie KA, wartość HbA1C podało 7 osób i wyniosła ona średnio $7,08 \pm 0,66\%$, a w grupie KC średnią wartość HbA1C podało 6 osób i wyniosła ona $6,92 \pm 0,95\%$ (Tab. II). Natomiast wśród uczestników badania udzielających odpowiedzi na pytanie odnoszące się do wartości hemoglobiny glikowanej podczas korzystania z systemu Nightscout w grupie A 3 osoby nie pamiętały dokładnej wartości, jakkolwiek 2 uczestników badania zaznaczało, iż była ona niższa niż przed rozpoczęciem korzystania z systemu. Tylko 1 osoba w tej grupie zaznaczyła, że jest ona wyższa. Wśród dorosłych uczestników korzystających z systemu Nightscout (grupa A) wartość HbA1C istotnie zmniejszyła się u 75% ($n = 15$) badanych, u 15% ($n = 3$) zwiększyła się, a u 10% ($n = 2$) pozostała bez zmian, przy czym różnice te były znamienne statystycznie ($p < 0,001$). W grupie A średnia wartość HbA1C przed korzystaniem z systemu Nightscout wynosiła $6,63 \pm 0,61\%$ i uległa istotnemu ($p = 0,007$) obniżeniu do $6,11 \pm 0,49\%$ podczas użytkowania systemu Nightscout.

W grupie C, korzystającej z systemu Nightscout u 84,8% ($n = 39$) uczestników wartość HbA1C zmniejszyła się, u 8,7% ($n = 4$) wzrosła, a u 6,5% ($n = 3$) pozostała bez zmian, przy czym różnice te były statystycznie istotne ($p < 0,001$). Wynik ten potwierdzają także kolejne analizy. Za pomocą testu znaków rangowanych Wilcozona porównano ze sobą średnie wartości HbA1C w grupie C przed rozpoczęciem stosowania Nightscout i podczas stosowania tego systemu. Średnia wartość HbA1C przed rozpoczęciem korzystania z systemu Nightscout wynosiła $6,67 \pm 0,76\%$, a podczas korzystania obniżyła się i wynosiła $6,15 \pm 0,51\%$ ($p = 0,011$).

Oceniając wyrównanie metaboliczne przed rozpoczęciem korzystania z systemu Nightscout, nie stwierdzono istotnych różnic między grupami A i KA, jak również C i KC.

BEZPIECZEŃSTWO

Na pytanie o częstość występowania hipoglikemii podczas korzystania z systemu Nightscout dorośli najczęściej wybierali odpowiedź, iż występują one rzadziej w stosunku do okresu przed korzystaniem z systemu Nightscout ($n = 16$; 18,2%), przy czym różnica ta była znamienna statystycznie ($p < 0,001$).

W grupie C od chwili użytkowania systemu Nightscout hipoglikemie zdarzały się rzadziej u 30 badanych (61,2%), a u 2 dzieci (4,08%) nie występowały wcale, podczas gdy wcześniej odnotowano tego typu incydenty. Różnice te były znamienne statystycznie ($p < 0,001$). U 8 dzieci (16,32%) użytkowanie systemu Nightscout nie wpłynęło na częstość występowania hipoglikemii, ponieważ zdarzały się one

porównywalnie często, a u 1 dziecka (2,04%) incydenty te nie występowały wcale. Tylko niewielka liczba dzieci ($n = 3$; 6,12%) zadeklarowała częstsze występowanie epizodów hipoglikemii w trakcie korzystania z systemu Nightscout. Troje badanych w tej grupie nie udzieliło w ogóle odpowiedzi.

W grupie A średnia tygodniowa liczba incydentów hipoglikemii przed korzystaniem z systemu Nightscout wynosiła $7,70 \pm 5,51$ incydentów i była znamienne statystycznie ($p < 0,001$) wyższa w porównaniu do liczby epizodów hipoglikemii występującej podczas korzystania z systemu Nightscout, która wynosiła $3,45 \pm 2,70$ epizodów na tydzień (Tab. III).

W grupie C wartości te wyniosły odpowiednio $5,51 \pm 3,69$ / tydzień przed oraz $2,57 \pm 2,30$ / tydzień podczas korzystania z systemu Nightscout i różniły się znamienne statystycznie ($p < 0,001$) (Tab. III).

W grupie KA średnia tygodniowa liczba incydentów hipoglikemii wynosiła $4,44 \pm 3,43$, a w grupie KC wartość ta wyniosła $6,90 \pm 6,79$ (Tab. III).

CIĘŻKIE HIPOGLIKEMIE

W grupie A, liczba epizodów ciężkich hipoglikemii przebiegających z utratą przytomności i koniecznością podania dodatkowych leków była wyższa przed rozpoczęciem korzystania z systemu Nightscout i wystąpiła u 3 osób, przy czym u 1 badanego incydenty te wystąpiły dwukrotnie, a u 2 jednokrotnie (Tab. III). Natomiast w trakcie korzystania z systemu Nightscout w grupie A nie odnotowano ani jednego incydentu tego typu. W grupie C incydenty te również nie występowały wcale, ale zdarzały się w grupie kontrolnej KC – u jednego dziecka wystąpiły trzykrotnie (Tab. III). Epizody ciężkich hipoglikemii znacznie częściej występowały w grupie kontrolnej dorosłych KA ($n = 5$) w liczbie od 1 do 5, w porównaniu do grupy A, jakkolwiek różnice te nie były znamienne statystycznie (Tab. III).

NIEŚWIADOME HIPOGLIKEMIE

Incydenty nieświadomych hipoglikemii nigdy nie wystąpiły u 11 osób z grupy A (Tab. III). U 1 osoby z tej grupy występowały wcześniej tego typu epizody, ale nie obserwowano ich w trakcie korzystania z systemu Nightscout. Natomiast u 8 dorosłych z grupy A incydenty te występowały nadal.

W grupie C u 25 dzieci nigdy nie występowały nieświadome hipoglikemie, u 5 dzieci z tej grupy podczas korzystania z systemu Nightscout nie powtórzyły się wcześniej występujące epizody nieświadomych hipoglikemii, natomiast u 19 badanych epizody te występują nadal.

Powyżej opisane różnice nie były jednak znamienne statystycznie zarówno u dorosłych, jak i u dzieci.

INNE POWIKŁANIA

Najczęściej zgłaszanym powikłaniem występującym przed rozpoczęciem korzystania z systemu Nightscout, oprócz jatrogennej hipoglikemii, była kwasica ketonowa, którą odnotowano u 3 dorosłych oraz u 5 dzieci (Tab. III). Nie stwierdzono natomiast występowania tego powikłania

Tabela II. Ocena stopnia wyrównania metabolicznego cukrzycy

Dorośli			
Rodzaj grupy (korzystanie z Nightscout)	A		KA
	przed	w trakcie	
HbA _{1c} (%)	6,63 ± 0,61	6,11 ± 0,49 ¹	7,08 ± 0,66
Dzieci			
Rodzaj grupy (korzystanie z Nightscout)	C		KC
	przed	w trakcie	
HbA _{1c} (%)	6,67 ± 0,76	6,15 ± 0,51 ²	6,92 ± 0,95

1 – p=0,007 (analiza wartości przed do wartości w trakcie)

2 – p=0,011 (analiza wartości przed do wartości w trakcie)

Tabela III. Bezpieczeństwo chorych przed i w trakcie korzystania z systemu Nightscout oraz grupa kontrolna

Dorośli			
Rodzaj grupy (korzystanie z Nightscout)	A		KA
	przed	w trakcie	kontrola
Hipoglikemia (liczba incydentów/tydzień)	7,70 ± 5,51	3,45 ± 2,70 ¹	4,44 ± 3,43
Ciężka hipoglikemia (liczba incydentów)	3 osoby (1-2 x)	0	5 osób (1-5 x)
Nieświadome hipoglikemie (nigdy/obecnie nie/nadal)	11 / 1 / 8		5 / 2 / 2
Kwasica ketonowa (liczba incydentów)	3	0	0
Dzieci			
Rodzaj grupy (korzystanie z Nightscout)	C		KC
	przed	w trakcie	
Hipoglikemia (liczba incydentów/tydzień)	5,51 ± 3,69	2,57 ± 2,30 ¹	6,90 ± 6,79
Ciężka hipoglikemia (liczba incydentów)	0	0	1 osoba (3 x)
Nieświadome hipoglikemie (nigdy/obecnie nie/nadal)	25 / 5 / 19		3 / 2 / 5
Kwasica ketonowa (liczba incydentów)	5	2	0

1 – p<0,0001 (analiza wartości przed do wartości w trakcie)

w obu grupach kontrolnych. Podczas korzystania z systemu Nightscout rzadziej występowały epizody kwasicy ketonowej, które obserwowano tylko u 2 dzieci, jednak nie były to różnice istotne statystycznie (Tab. III).

ZALETY

W ocenie ankietowanych najbardziej istotną korzyścią związaną z korzystaniem z systemu Nightscout był wpływ na bezpieczeństwo (n=76, 86,4%). Na 2 miejscu wybierano możliwość lepszej kontroli glikemii i wyrównania metabolicznego (n=70, 79,5%). Z kolei w odpowiedzi otwartej podawano, że w systemie tym cenne są: poprawa komfortu życia (n=7, 8%) oraz umożliwienie korzystania z systemu zamkniętej pętli „AndroidAPS” (n=3, 3,4%). Dwóch ankietowanych podało, że korzystanie z systemu Nightscout było warunkiem uczęszczania dziecka do przedszkola.

WADY

W trakcie korzystania z systemu Nightscout uczestnicy badania najbardziej skarżyli się na przemijające problemy techniczne (n=34, 38,6%). Niewiele mniej osób wskazywa-

ło na brak poparcia ze strony towarzystw naukowych (n=27, 30,7%) oraz brak firmowego wsparcia (n=25, 28,4%). Do najczęściej wymienianych niedogodności systemu Nightscout w odpowiedzi otwartej należały: uciążliwy proces instalacji (n=3, 3,4%) oraz konieczność posiadania dodatkowego, niefabrycznego urządzenia – uploadera (n=5, 5,7%).

ODCZYT WYNIKÓW

Zdecydowana większość użytkowników systemu Nightscout n=68 (77,3%) odczytywała wyniki na smartfonie. W następnej kolejności były to komputer (n=50, 56,8%) oraz smartwatch (n=31, 35,2%). Większość ankietowanych dokonywała odczytu na więcej niż jednym urządzeniu (n=52, 59,1%). Ponadto niektórzy respondenci n=5 (5,7%) wykorzystywali do tego celu tablety (n=5, 5,7%), a nawet telewizory (n=2, 2,3%).

ZAMKNIĘTA PĘTLA

Pojęcie „zamkniętej pętli” nie było znane tylko niewielkiej części badanych n=11 (12,5%). Zdecydowana większość ankietowanych (n=65, 73,9%) słyszała o tym rozwiązaniu,

natomiast 12 ankietowanych (13,6%) korzysta z tego rozwiązania w swojej osobistej pompie insulinowej, z tego 5 dorosłych oraz 7 dzieci.

DYSKUSJA

Zgodnie z wiedzą autorów w chwili obecnej jest to pierwsze badanie dotyczące diabetyków i ich opiekunów korzystających z systemu Nightscout przeprowadzone na polskiej populacji. Pierwszy raz projekt Nightscout poddano ocenie za pomocą anglojęzycznej anonimowej ankiety przeprowadzonej w grupie „CGM in the Cloud” [14, 15]. W cytowanej artykule wysunięto wnioski, iż osoby korzystające z systemu Nightscout osiągały lepsze wyrównanie metaboliczne oraz poprawiła się ich jakość życia. Analiza przeprowadzonych ankiet potwierdziła korzystny wpływ systemu Nightscout na bezpieczeństwo poprzez redukcję hipoglikemii oraz obniżenie hemoglobiny glikowanej. W badaniu wykazano również redukcję epizodów ostrego powikłania cukrzycy pod postacią kwasicy ketonowej.

Projekt Nightscout został zaprojektowany przez pacjentów – dla pacjentów. W odróżnieniu od komercyjnych rozwiązań nie posiada on firmowego wsparcia technicznego. Osoby pragnące uruchomić system czy też użytkować go na co dzień muszą posiadać pewne zdolności informatyczne. Problem ten w pytaniach otwartych opisywali ankietowani. Obawiali się tego, iż nie podołają procesowi instalacji z powodu zbyt małego doświadczenia i umiejętności informatycznych. Mimo braku wsparcia firmowego, użytkownicy nie są pozostawieni sami sobie. Członkowie zrzeszeni w grupie internetowej często rozwiązują problemy techniczne wspólnie oraz wymieniają się nowymi rozwiązaniami technologicznymi. Ponadto w internecie dostępne są samouczki oraz instrukcje instalacji.

Korzystanie z systemu Nightscout jest bezpłatne. Niemniej jednak użytkownik musi posiadać stały dostęp do internetu oraz utworzone konto na serwerze danych. Ponadto, aby móc pobrać dane z systemu ciągłego monitorowania glukozy konieczne jest posiadanie uploadera (w zależności od używanego systemu CGM uploadery różnią się pomiędzy sobą). Koszt wspomnianego urządzenia jest relatywnie niewielki, w większości to kilkaset złotych, a zakup jest jednorazowy.

Znaczna część użytkowników systemu Nightscout, którzy brali udział w badaniu to dzieci i młodzież. Należy pamiętać, że system Nightscout powstał, aby ułatwić rodzicom nadzór nad ich podopiecznymi, gdy nie ma ich w pobliżu. Jednak oprócz dzieci z systemu korzystają osoby dorosłe. Dane zapisane na zewnętrznych serwerach pozwalają w dowolnym czasie i miejscu dokonać wglądu zarówno do aktualnych wartości glikemii, jak i do wcześniejszych pomiarów. Daje to możliwość opracowania statystycznego i może stanowić elektroniczną formę notatnika glikemii, będąc ułatwieniem dla diabetyka oraz lekarza prowadzącego.

12 uczestników przeprowadzonego badania korzystało z systemu zamkniętej pętli w swojej osobistej pompie insulinowej. Jest to rozwiązanie, które automatycznie zwiększa

podaż insuliny w przypadku hiperglikemii. Korzystanie z systemu Nightscout dostarcza cennych informacji algorytmowi odpowiedzialnemu za dostosowanie aktualnej dawki insuliny. System Android APS podobnie jak projekt Nightscout jest rozwiązaniem stworzonym przez pacjentów [16]. Na zwiększającą się liczbę użytkowników systemu DIY AndroidAPS oraz konieczność wspomagania osób decydujących się na takie rozwiązanie zwracają uwagę autorzy stanowiska Szkoły Pompowej Polskiego Towarzystwa Diabetologicznego [17].

Przeprowadzone badanie wykazuje szereg ograniczeń. Było ono przeprowadzone za pomocą anonimowej ankiety internetowej, a część pytań miała charakter retrospektywny i odnosiła się do czasu sprzed rozpoczęcia korzystania z systemu Nightscout. W pytaniu o wartość HbA1C respondenci, którzy nie pamiętali dokładnej liczby, mogli wybrać jedynie odpowiedź porównawczą. Jednak badani, którzy podali dokładną odpowiedź mogli ją przeszacować, podobnie jak częstość epizodów hipoglikemii. Warto zaznaczyć, że grupa badana nie była zbyt liczna z uwagi na: niewielkie rozpowszechnienie tej formy udoskonalenia standardowych pomiarów śródkankowego stężenia glukozy, określony przedział czasowy wypełniania ankiety i ograniczoną liczbę osób zrzeszonych w grupie na portalu społecznościowym. Uzyskane przez nas wyniki sugerują konieczność dalszego badania rozwiązań DIY projektowanych przez pacjentów oraz analizowanie ich pod względem skuteczności i bezpieczeństwa leczenia cukrzycy.

WNIOSKI

1. Korzystanie z systemu Nightscout może pozytywnie wpływać na bezpieczeństwo stosowanej insulinoterapii oraz proces leczenia cukrzycy typu 1.
2. System Nightscout może być udoskonaleniem systemu ciągłego monitorowania śródkankowego stężenia glukozy.

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ORIGINAL ARTICLE

CONDITIONS OF MICROELEMENTS EXCHANGE PROCESSES IN WOMEN'S PLACENTS IN INTRAUTERINE INFECTION OF THE FETUS

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ABSTRACT

The aim: to study the content of trace elements (Fe, Cu, Zn, Co, Cr, Ni, Pb) in the placenta of pregnant healthy women and with signs of intrauterine infection of the fetus, features of transplacental transmission of infectious process from mother to fetus and to investigate the role of the placenta in trace element supply of the fetus.

Materials and methods. 43 pregnant women between the aged 16 to 40 years were monitored, including 12 with physiological pregnancy (group 1) and 31 with signs of STI (group 2). All pregnant women underwent standard comprehensive examination, evaluation of fetal cardiac output and non-stress testing using cardio-tocography (CTG) in the third trimester. The group of pregnant women with signs of fetal ulcers included women whose pregnancy was complicated by chronic fetoplacental dysfunction (FPD), infectious lesions of the fetoplacental complex, which were diagnosed on the basis of ultrasound signs of placenta, syndrome of infectious and surrounding infections.

Results: The content of essential trace elements in the placenta of the main group was significantly lower than in the placenta of the control group. There was a decrease in the concentration of iron by 32%, zinc – by 46%, nickel – by 44%, copper more than tripled, chromium – 4 times. Deficiency of essential trace elements (iron, zinc, copper, chromium, nickel) and elevated lead content in the placenta leads to the formation of conditions for the development of placental dysfunction, the progression of which leads to fetal distress, developmental delay syndrome and antenatal fetal death.

Conclusions: 1. One of the links in the pathogenesis of intrauterine infection in the fetus is the imbalance of essential trace elements in the system «mother – placenta – fetus». 2. Pregnant women with signs of intrauterine infection are characterized by a deficiency of serum Fe, Cu, Zn, Ni and an increased content of Pb, Cr and Co compared with pregnant women with physiological pregnancy. 3. Umbilical cord blood of women with evidence of fetal fetal infection also has a reduced content of iron, copper, zinc and high levels of lead, cobalt and chromium. 4. Disruption of placental function in intrauterine infection of the fetus is caused by reduced content of iron, zinc, copper, nickel and lead accumulation.

KEY WORDS: trace elements, placenta, intrauterine infection of the fetus

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INTRODUCTION

The problem of intrauterine infection is one of the leading obstetric practices. According to the Ministry of Health of Ukraine, only 6% of practically healthy children are born in our country today, every third child has a physical or mental health disability, and every second is a carrier of cytomegalovirus, herpes and other infections. Infectious pathology is diagnosed in 50–60% of hospitalized full-term and 80% of premature infants [1]. According to the results of pathoanatomical studies, in 38% of deceased children, infection is the main cause of death or concomitant nosology, which complicates the course of the underlying disease [1, 2].

The basis of the processes of interaction between mother and fetus organisms during pregnancy is the formation of a single functional system «mother-placenta-fetus». The placenta is a link in the formation of a complex that promotes the preservation and progression of pregnancy. In intrauterine infection (IUD) of the fetus, various medical, environmental, social, biological factors are combined,

accompanied by metabolic disorders, imbalance of trace elements metabolism, development of tissue hypoxia in the mother and fetus [3, 4]. In the vast majority of women, there are significant shifts in the immune, coagulation, neuroendocrine systems, which correlates with changes in the fetus and tissues of the fetoplacental complex (FPC), especially the placenta [5, 6]. The first reactions of the body in response to the development of trace elements are manifested in the preclinical stages. Temporarily latent pathological process is manifested by pseudoadaptation syndrome, then by maladaptation syndrome of the mother and FPC. In its clinical manifestations include transient hypertonicity of the uterine body, placental hyperplasia, chronic fetal hypoxia.

The progression of this process leads to dysfunction, premature aging of the placenta, delayed fetal growth and other perinatal complications [7, 8, 9].

Essential trace elements (MEs), which include zinc, iron, cobalt, chromium, copper, manganese, which ensure the functioning of the placenta, membrane permeability, active

Table II. The content of trace elements in the placenta

ME	The placenta of women with signs of fetal IUD, mcg / g	The placenta of women with physiological pregnancy, mcg / g	p < 0,05
Fe	14439,0 ± 3094,0	21267,83 ± 1262,93	p < 0,05
Zn	2748,65 ± 184,43	5093,73 ± 437,31	p < 0,001
Cu	96,93 ± 11,04	309,58 ± 77,58	p < 0,01
Co	164,45 ± 39,72	64,53 ± 15,57	p < 0,05
Cr	43,51 ± 7,46	169,41 ± 49,49	p < 0,05
Ni	44,83 ± 4,94	78,0 ± 12,2	p < 0,05
Pb	72,16 ± 14,97	37,08 ± 5,7	p < 0,05
Mn	0,059 ± 0,011	0,041 ± 0,05	–

transport of chemicals, fetal oxygenation, energy and other types of metabolism. Their antagonists are heavy metals that can bind to amino acids, enzymes, nucleic acids, SH-groups of blood cell proteins [10, 11]. Heavy metal cations significantly affect the functional state of cell mitochondria, disrupting their metabolism and membrane permeability, which determining the activity of enzymes. Suppression of the activity of enzymes of biological oxidation by toxic metal inhibitors or damage to the membrane structures of mitochondria and other cellular elements is accompanied by hemocoagulation disorders with impaired blood coagulation system, and decrease ability of the placenta to absorb oxygen. These phenomena are one of the causes of tissue hypoxia and disorders of the compensatory-adaptive mechanisms in the placenta and fetoplacental complex as a whole [12, 13].

THE AIM

To study the content of trace elements (Fe, Cu, Zn, Co, Cr, Ni, Pb) in the placenta of healthy pregnant women with signs of intrauterine fetal infection, features of transplacental transmission of infectious process from mother to fetus and to investigate the role of the placenta in trace element supply of the fetus.

MATERIALS AND METHODS

The study was conducted at the Sumy Regional Clinical Perinatal Center and the Department of Obstetrics and Gynecology of Sumy State University during 2012-2018. In total, 43 pregnant women aged 16 to 40 years were monitored, including 12 with physiological pregnancy (group 1) and 31 with signs of STI (group 2). All pregnant women underwent standard comprehensive examination, evaluation of fetal cardiac output and non-stress testing using cardio-tocography (CTG) in the third trimester. Ultrasound was performed by transabdominal scanning using an Aloka SSD-1800 ultrasonic portable scanner (Toshiba, Japan) with a 3.5 to 10 MHz probe. When determining the pathological biophysical profile of the fetus (BPP), Doppler blood flow velocity was performed in the umbilical artery,

the middle cerebral artery. This study was approved by the ethics committee of the Sumy State University, Ukraine. All procedures were carried out in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

The group of pregnant women with signs of fetal ulcers included women whose pregnancy was complicated by chronic fetoplacental dysfunction (FPD), infectious lesions of the fetoplacental complex, which were diagnosed on the basis of ultrasound signs of placenta, syndrome of infectious and surrounding infections.

The content of trace elements (Fe, Cu, Zn, Co, Cr, Ni, Pb) was determined by the method of atomic absorption spectrophotometry on the C-115M1 spectrophotometer manufactured by NGO Selmi (Ukraine). The material for the study was taken from central part of placenta immediately post delivery of which, a section of tissue was cut out, pre-determining the weight, burned, and ash was obtained. The arithmetic mean (M) and the arithmetic mean (m) were determined for all indicators. Using Student's t test (t), the confidence index (p) was determined and the correlation (r) was calculated.

RESULTS AND DISCUSSION

The content microelements of in the placenta of women with signs of fetal intrauterine infection compared with the content in the placenta of women with physiological pregnancy had its differences. Thus, the content of essential trace elements in the placenta of the main group was significantly lower than in the placenta of the control group. There was a decrease in iron concentration by 32% (p < 0.05), zinc – by 46% (p < 0.001), nickel – by 44% (p < 0.05), copper more than three times (p < 0.01), chromium – 4-fold (p < 0.05) (Table I).

Reducing the content of vital microelements indicates that infectious tissue damage leads to the partial release of trace elements from cells through damaged membranes. This mechanism is possible in inflammatory and necrotic processes in the placental tissue. The exceptions were cobalt

and manganese, whose contents were 2.5 ($p < 0.05$) and 1.5 times higher in the placenta of the main group, respectively. These metals are known as activators of redox reactions, a number of enzymes. In our opinion, the accumulation of manganese and cobalt in the placenta of women with signs of fetal infection is associated with the activation of compensatory responses.

The quantitative determination of the toxic content of lead in the placenta showed that the fetal lead was 50% significantly higher ($p < 0.05$) than in the control group. Intense deposition of lead in the placenta on the background of fetal IUD has a negative effect on the respiratory, trophic, protective functions of the placenta. This phenomenon contributes to the increase in the frequency of fetoplacental insufficiency and infectious defeat of the fetoplacental complex.

When studying the content of IU, in order to reduce their concentration in the placenta of women with physiological pregnancy, the arrangement was as follows: Fe > Zn > Cu > Cr > Ni > Co > Pb > Mn.

In the placenta of women with signs of fetal IUI, the location of the IU was as follows: Fe > Zn > Co > Cu > Pb > Ni > Cr > Mn.

In the main group, there is a direct (strong) correlation: for Zn, with Ni; for Cr – with Co, Pb; for Mn – with Cu ($p < 0.001$). A direct correlation of mean strength was found for Zn – with Pb, Cu, Co; for Mn – with Co; for Co – with Pb; for Cu with Pb ($p < 0.01$). We observe a weak (direct) correlation for Cr with Co; for Ni – with Cr ($p < 0.05$). No correlation was found between these elements, which could indicate a membrane-toxic and enzymatic effect and impaired structure and function of lining tissue cells and determine the fetal condition of the newborn. From the above it shows that the deficiency of essential trace elements (iron, zinc, copper, chromium, nickel) and the increased content of toxic ME of lead in the placenta leads to the formation of conditions for the development of placental dysfunction, the progression of which leads to fetal distress, developmental delay syndrome and fetal death. In the physiological course of pregnancy, it is possible to assume that there are certain mechanisms in the placenta for protection against toxic ME and preservation of vital ME that ensure the satisfactory functioning of fetoplacental complex.

CONCLUSIONS

1. One of the links in the pathogenesis of fetal intrauterine infection is the imbalance of essential trace elements in the system «mother – placenta – fetus».
2. Pregnant women with signs of intrauterine infection are deficient in serum iron, copper, zinc, nickel, and elevated lead, chromium and cobalt content compared to pregnant women with physiological pregnancy.
3. Umbilical cord blood of women with evidence of fetal infection also has a reduced content of iron, copper, zinc and high levels of lead, cobalt and chromium. Therefore, the deficiency of essential trace elements in the body of a pregnant woman is accompanied by their deficiency in the body of the fetus.

4. Disruption of placental function in intrauterine infection of the fetus is caused by reduced content of iron, zinc, copper, nickel and lead accumulation.

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ORIGINAL ARTICLE

CURRENT STATE OF STUDENTS' HEALTH AND ITS IMPROVEMENT IN THE PROCESS OF PHYSICAL EDUCATION

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ABSTRACT

The aim is to investigate the state of health of the students of Ukrainian higher education institutions.

Materials and methods: The study of state of health was conducted at Polissia National University and Zhytomyr Ivan Franko State University in 2008–2019. The results of a medical examination of 3737 students of different faculties between the ages of 17 and 22 were examined. Medical examinations were conducted by the doctors of the medical centers of universities. Totally 1208 pupils (male and female) of the schools of Zhytomyr region were involved in the questionnaire, which was conducted to analyze the attendance of physical education classes by pupils while studying at school. 648 students of Polissia National University participated in the study of the self-assessment of their own health, causes, and structure of disease incidence, the dynamics of the loss of labor capacity.

Results: It was established that from 24 to 30.8 % of the students had diseases and a low level of physical development that indicated a low level of physical education at schools. It was revealed that the number of sick students was increasing during the study at higher educational institutions (HEI) that led to missing classes. Besides, up to 35.5 % of students had health disorders and belonged to a special medical group. And 1.7 % of the students had chronic diseases and disabilities and were exempted from sports. It was found that physical education and sports took the 5th place in the system of life values of male students and the 12th place in the system of life values of female students.

Conclusions: Physical education, aimed at enhancing the functional capacity of an organism, the development of physical qualities, the improvement of the special physical and technical readiness in the sphere of physical activity types and the acquisition of professionally applied skills and abilities, is the basis for strengthening the health of students of Ukraine.

KEY WORDS: health, diseases, physical education, students

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INTRODUCTION

The problem of health has always been one of the most pressing problems both at the level of scientific cognition and at the level of everyday consciousness [1, 2, 3]. Considering the modern integrated interdisciplinary concept of health, it combines the psychological and physiological aspects of human health. The integral concept is not the only one among the theories of health, because health is studied by researchers in different scientific disciplines, including medicine, physiology, anatomy, psychology, ecology, pedagogy, and others [4, 5, 6]. Individual health is essential in the strategy and tactics of health promotion, preservation, and restoration, where individual, age, gender, constitutional, regional, and social health indicators are highlighted [7, 8].

Complete health is a rare condition for most people in general. However, young people, who are students, have higher resiliency, no discomfort, their mood and well-being are good, they are full of vigor and strength, and a careful examination can show no definite abnormality [9, 10]. However, the majority of students usually do not have such vivid manifestations of health, well-being is a subjective characteristic [8, 11]. Much of the scientists consider the term “health” as a condition of an organism that enables a person to implement the genetic program in the specific conditions of the socio-cultural existence of a human to the maximum extent [12, 13].

The concept of “health” of the population is characterized by a set of indicator, including demographic one (birth and

deaths rates, average life expectancy), physical development (morphofunctional and biological development, harmony), disease incidence (general, infectious), disability (primary, general), the quality of donozological states (immunity, enzyme activity) [14, 15, 16]. The research [17] showed that the majority of freshmen (74.3 %) belonged to the main medical group, i.e. they were healthy. But 17.3 % had various diseases, and 1.04 % were exempted from sports, they had disabilities or were significantly sick. While studying at HEI, the number of students in the main group was reduced by 8.1 %, while the number of students in the special group was increased by 7.2 %. The number of students exempted from sports had been reduced by the 2nd year of study and after that, there was a tendency of a slow increase in their number in the 3rd year. Thyroid hyperplasia had been in the first position for three years among males. The second most prevalent group was digestive diseases, the third group included respiratory diseases [18]. The largest group consisted of acute respiratory diseases, including angina, influenza, chronic bronchitis, pneumonia, bronchial asthma. The number of diseases of the nervous system, sensory organs, and musculoskeletal system was increased. This resulted in an overall increase in disease incidents of the students by 9.6 % [19].

According to research by E. Kotov [20], the majority of the 1st and 2nd-year students belonged to the main medical group. At the same time, 15-20 % of young people had health disabilities. The author noted that the number of students was constantly decreasing in the process of study in the main medical group, and increasing in the preparatory and special ones. From the first to the fourth year of study the number of students in the main medical group decreased by 22.5 % among males and 17.5 % among females.

The results of the research [21] showed that 37.2 % of students of the 1st – 4th years of study had a middle level of health indicators, 20.4 % – lower than the middle, 13.2 % – low, 15.6 % – higher than the middle, 12.0 % – high. There was also an interconnection between somatic health and students' activity.

According to the Ukraine-wide monitoring of the Institute of Sociology NAS of Ukraine, 58 % of young people (up to 30 years of age) stated that they were in good health, moreover, 36.4 % of them rated their health as good and 47.9 % – as satisfactory. Young people were mainly satisfied with their health and it was not a meaningful value for them [22].

As a result of annual medical examinations of the students from different HEI of Ukraine, it was found that 70-80 % of freshmen had health disabilities [14, 15, 23]. Most of the diseases that made the students miss classes were cold-related diseases. There was also a direct correlation between an increase in the number of students who had a low level of physical fitness, and deterioration of somatic health, especially in endurance and speed tests [12, 21]. Such motor qualities as general endurance, speed, agility, strength endurance, and flexibility are beyond the safe level of somatic health, indicating a direct correlation between health and physical fitness [24]. This peculiarity can be traced concerning almost all motor qualities, except

upward jump and standing long jump that characterize such motor quality as speed force [25].

According to the research [26, 27, 28], the level of physical health, which was calculated on the basis of indicators that have correlative relationships with maximum aerobic capacity and characterize the bioenergy of the body, was lower than the middle during the period of study. None of the students had a high level of health, and only 2-4 % of students had higher than the middle level. The index of the physical condition during study decreased from the middle level of the 1st – 2nd grades to the lower than the middle in the 3rd – 4th years of study. The authors also examined that 72 % of the surveyed students had chronic diseases, the incidence rate was 112.5 ± 6.2 cases per 100 students (some students were determined to have 2-3 diseases), and the rate of the females was 1.4 times higher than the one of males. The diseases of the nervous system and sensory organs (37.8 ± 2.7 %), the diseases of the endocrine system, eating disorders and metabolic disorders (35.3 ± 2.6 %) took the leading places in the structure of the incident rate.

The scholars [29, 30] note that the level of students' health depends significantly on the state of physical education at school, following a healthy lifestyle by school pupils, and the prevention of diseases among pupils, that is, on the initial level of health of a HEI applicant. There has been a significant deterioration of health and a decrease in the level of fitness of young students in Ukraine for many years [31, 32]. The examination of the physical development and physical fitness of the students of Polissya National University for thirty years showed that more than 50 % of them could not pass physical tests with "satisfactory results". They had significant physical disabilities, diseases; from 10.9 to 20.5 % belonged to the preparatory group, from 8.1 to 17.4 % – to a special medical group, and from 0.4 to 1.2 % were exempted from sports, had chronic diseases and disabilities [21].

The analysis of literary sources leads to the conclusion that health is determined as the interaction of many factors – social and biological, external and internal, material and spiritual, which interact with each other in a complicated and contradictory manner. Undoubtedly, health prevention is also significantly influenced by physical activity, i.e. physical education at schools and educational establishments. The quality of the educational process and the students' personally-oriented choice of physical activity depend on the health state of young people in Ukraine.

THE AIM

The aim of the study is to investigate the state of health of the students of higher educational institutions of Ukraine.

MATERIALS AND METHODS

The study of state of health was conducted at Polissya National University (PNU) and Zhytomyr Ivan Franko State University (ZSU) in 2008-2019. The results of a medical examination of 3737 students of different faculties be-

tween the ages of 17 and 22, including 1929 students of PNU and 1808 students of ZSU, were examined. Medical examinations were conducted by the doctors of the medical centers of universities. Totally 1208 pupils (male and female) of the schools of Zhytomyr region were involved in the questionnaire, which was conducted to analyze the attendance of physical education classes by pupils while studying at school. 648 students of PNU participated in the study of the self-assessment of their own health, causes, and structure of disease incidence, the dynamics of the loss of labor capacity.

Research methods: theoretical (the analysis and synthesis of literary sources, the analysis of curricula, the assessment of the quality of classes with students of special medical groups, the study and analysis of medical cards of students); empirical (pedagogical observations, questionnaires, the methods of mathematical statistics).

This study complies with the ethical standards of the Act of Ukraine "On Higher Education" No.1556-VII dated 01.07.2014 and the Letter from the Ministry of Education and Science of Ukraine "On the Academic Plagiarism Prevention" No. 1/11-8681 dated 15.08.2018. Also, this study followed the regulations of the World Medical Association Declaration of Helsinki – ethical principles for medical research involving human subjects. Informed consent was received from all individuals who took part in this research.

RESULTS

The level of physical fitness and health of students while studying at HEI is significantly dependent on the physical condition of university applicants. The analysis of physical fitness, physical development and disease incidence of applicants showed that from 24 to 30.8 % of them had diseases, including chronic, congenital anomalies, disability, very poor physical development (postural disorders, overweight or underweight, disproportionate physical development of the body, problems with the functionality of the body, etc.). Performing tests in physical education and assessing physical development, many students do not have technical skills or knowledge about the viability of such measurements and tests. This condition of the applicants' preparation substantially depends on the state of physical education classes and health-improving activities in Ukrainian schools. There are number of disadvantages of attending physical education scheduled and extracurricular classes at high school. There are some pupils from both rural and urban schools who did not attend physical education classes at all. We defined that 71.7 % of males and 82.7 % of females among the youth from rural areas and 47.1 % of males and 75 % of females among the youth from urban areas who did not attend any extracurricular activities in sections, sports schools, and clubs. And 50.7 % of males and 75.6 % of females from the rural area and 55.5 % of males and 69.6 % of females from the city did not do physical exercise themselves while studying at school (Table I).

This state of physical education at school will surely be reflected in the level of physical fitness and health of future

students. The problem of students' health and physical fitness level arises by far earlier the entry of an applicant to HEI and requires a comprehensive and systematic solution within the country but not in a particular region or institution. In addition, the industrial and technical development of the industry caused significant changes in the environment, accelerated the rhythm and pace of life, computerization increased the nervous-emotional tension against the background of a significant decrease in motor activity, which contributed to the emergence of cardiovascular diseases, the diseases of the respiratory and musculoskeletal system, etc. Diseases are significantly reflected in the decline in students' performance and its complete loss. Our research during the calendar year, which included 648 students of PNU, showed that students lost 4.45 % of school days out of the total number of days because of illness just in a year. The most common are cold-related diseases that make students lose their working capacity. The next are the diseases of musculoskeletal, respiratory, cardiovascular and nervous systems and others. The analysis of the causes of the loss of labor capacity by university students showed significant differences between males and females not only in the number of diseases but also in their consequences. Thus, 2.18 % of first-year male students lost their working capacity during the year, the number of such female students accounted for 6.19 %, their number accounted for 2.69 and 4.48 % respectively in the 2nd year, and the situation was even more vulnerable in the 3rd and 4th year. There was a tendency to decrease in the loss of labor capacity of males, and a significant increase in the loss of labor capacity of females: 1.34 and 7.16 % in the 3rd year and 1.86 and 7.53 % in the 4th year (Fig. 1).

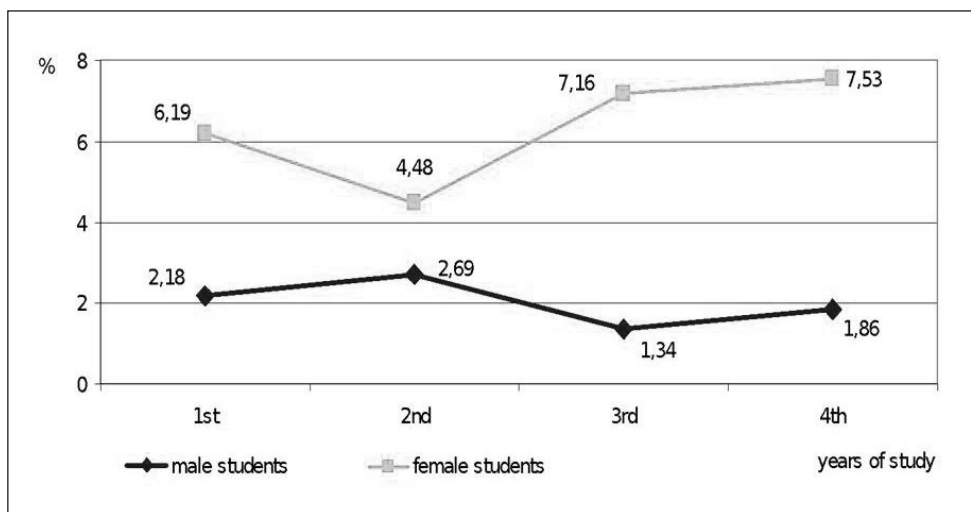
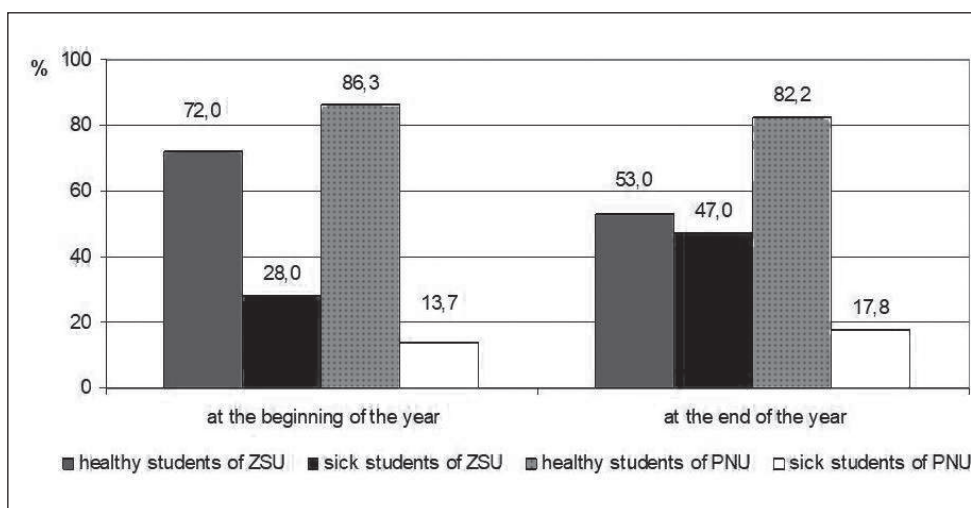
Further studies were aimed at examining students' health status, the dynamics of disease incidence over one academic year, and comparing the disease incidence of the students of two universities. The results of the study showed that 62.8 % of the students of ZSU and 82.2 % of the students of PNU were healthy and belonged to the main medical group for health reasons and could fulfill the program requirements for physical education and take tests in physical training. At the same time, 35.5 % of the students of ZSU and 17.8 % of the ones of PNU had disabilities and belonged to a special medical group. Among them, 1.7 % of the students of ZSU and 0.4 % of the students of NPU had chronic diseases, disabilities and were exempt from sports.

The analysis of the students' examination showed that the reason for the high disease incidence is living in radiation-contaminated territories. Thus, 10.3 % of the students of ZSU and 11.4 % of the students of PNU lived in the contaminated territories, often consuming foods and water from the area. In addition, we identified a number of major nutritional deficiencies in both universities that also contributed to poor health. The large learning load, stressful situations, poor sanitary and living conditions also affected the health of students.

The study found that in the first year of study, the number of sick students increased by 19 % at ZSU, and by only 4.8 % at NPU, where they took a number of additional health-im-

Table I. The analysis of pupils' attending physical education classes while studying at the school in senior year (n=1208, %)

Types of classes	Place of residence	Gender	Number of classes per week			
			3 and more	2	1	0
Scheduled classes	rural area	males	8.6	79.9	10.1	1.4
		females	3.9	83.5	10.2	2.4
	city	males	5.8	86.5	7.7	–
		females	2.7	90.1	6.6	0.6
Extracurricular classes in sections, sports schools, clubs	rural area	males	13.0	10.2	5.1	71.7
		females	3.2	3.9	10.2	82.7
	city	males	33.6	13.5	5.8	47.1
		females	11.4	9.8	3.8	75.0
Independent classes	rural area	males	20.3	15.9	13.1	50.7
		females	14.2	3.9	6.3	75.6
	city	males	18.7	11.6	14.2	55.5
		females	14.7	5.4	10.3	69.6

**Fig. 1.** The dynamics of the students' loss of labor capacity due to illness during the calendar year (% of the total number of days)**Fig. 2.** The dynamics of the students' health during one academic year

proving measures in the process of physical education and extra-curricular time and provided additional free food for many students (Fig. 2).

The study of the students' health allowed us to determine the structure of the diseases (n=648). Eye diseases associat-

ed with visual deterioration (myopia of different degrees) predominate in the structure of diseases of the students of the 1st – 2nd years – 28.5%. Thyroid disorders took second place, including hyperplasia of the 1st – 2nd degrees – 18.2 %, which can certainly be attributed to the consequences

Table II. The self-assessment of health state by the students of different years of study (n=647, %)

The state of health	Gender	The years of study				Total %
		1st	2nd	3rd	4th	
Perfect	males	5.7	9.4	8.6	2.9	6.7
	females	–	3.1	–	8.2	3.2
	in total	3.2	4.7	3.5	6.7	4.5
Good	males	60.0	67.1	57.1	50.0	60.1
	females	46.3	51.4	41.2	51.8	49.1
	in total	54.0	55.3	47.7	51.4	53.2
Satisfactory	males	30.5	21.9	17.1	32.4	26.5
	females	46.3	37.2	45.1	31.8	38.9
	in total	37.4	33.3	33.7	31.9	34.3
Poor	males	3.8	1.6	17.1	11.8	6.3
	females	6.2	7.3	13.7	8.2	8.1
	in total	4.9	5.9	15.1	9.2	7.4
Very poor	males	–	–	–	2.9	0.4
	females	1.2	1.0	–	–	0.7
	in total	0.5	0.8	–	0.8	0.6

of radiation. Besides, 13.5 % of the students were found to have disorders of the nervous system, 11.9 % – cardiovascular diseases, 6.5 % – diseases of the gastrointestinal tract. Some students were defined to have respiratory diseases (bronchial asthma, chronic bronchitis) – 3 %, scoliosis – 2.2 %, vegetative-vascular dystonia in combination with thyroid hyperplasia – 2.9 % and others. There were also cases of pancreatic diabetes, congenital anomaly of lower limbs, congenital deformity of eyes, etc.

The self-assessment of a health state by the students of NPU showed that only 6.7 % of males and 3.2 % of females indicated that they had a perfect state of health; 60.1 and 49.1 % respectively – a good state; 26.5 and 38.9 % – satisfactory; 6.3 % and 8.1 % – poor, and 0.4 and 0.7 % – very poor (Table II). Females, the majority of who belonged to specialized medical group, had lower physical development and physical fitness, rated their health slightly lower.

The analysis of the results of self-assessment by the students from different educational departments indicated significant differences between the educational departments. The self-assessment confirmed that there were no students with perfect health in the special education department, the number of students with perfect health accounts for 4.8 % of males and 4.5 % of females in the main department, 15.3 % of males and 1.5 % of females in the sports department. There were also differences found in the self-assessment of the students with good, satisfactory and poor health (Table III). It should be noted that 6.2 % of female students in the sports educational department confirmed a poor state of health despite engagement in sports. A very significant difference was also observed in the self-assessment of the students of the special education department concerning the poor and very poor health that proves the truth of such assessment.

The reduction of disease incidence and ensuring the efficient state of health of students is one of the priorities of the HEI physical education system. Therefore, it is necessary to create and maintain an environment that will contribute

to the formation of goals, value orientations, principles of healthy lifestyles and control over the factors that determine or influence the students' health in the university. The students' value orientations influence their purposefulness, life activity or passivity. The developed system of value orientations of the students encourages them to take health-improving measures, to follow a healthy lifestyle. The analysis made it possible to establish that among a large number of students' value orientations, health value got the highest desirability status and occupied a dominant position. At the individual level, the value of health was determined by many factors, among which the objective health of an individual took the first place. The obtained data gave good reason to claim that the "dominant nucleus" in the structure of the students' life values were their own health, the health of their parents, love, family and children, material values, education (career), i.e. they were ranked 1st – 5th. At the same time, other components of the value system, such as recognition by others, the environment protection, and the arts, occupied secondary places, that is, the last ones – 15th – 17th. Among the low ranked values were discipline, honesty, decency (males – 10, females – 8), independence (10 and 12 respectively), faith in God and spirituality (13, 12), peace of mind (15 and 11). It is interesting to note that such leading activities for students as physical culture and sports took only 9th place for males and 14th place for females (Table IV).

The system of value orientations of students is not solid. A student lives and develops in the conditions of a certain social environment, living conditions, education and upbringing, the system of values is changed from year to year, new values appear, the old ones are rethought and re-evaluated. The students' state of health, level of physical development and physical fitness, that is, belonging to a certain educational department have impact on the hierarchy of life values of the student's personality. Such life values as one's own health; parents' health; love, family and children; material values are priorities and occupied the

Table III. The self-assessment of health state by the students of different educational departments (n=647, %)

The state of health	Gender	Education departments		
		Special	Main	Sports
Perfect	males	–	4.8	15.3
	females	–	4.5	1.5
	in total	–	4.6	8.1
Good	males	41.6	69.0	59.3
	females	35.0	54.6	50.8
	in total	37.3	59.5	54.8
Satisfactory	males	35.6	23.0	25.4
	females	51.6	33.2	41.5
	in total	46.1	29.8	33.9
Poor	males	20.6	3.2	–
	females	12.4	6.9	6.2
	in total	15.3	5.6	3.2
Very poor	males	1.9	–	–
	females	1.0	0.8	–
	in total	1.3	0.5	–

Table IV. The hierarchy of life values of the students of Zhytomyr region (n=648, rank)

Life values	Gender	The year of study				Overall rating
		1st	2nd	3rd	4th	
Health	males	1	1	1	1	1
	females	1	1	1	1	1
Parents' health	males	3	3	3	2	3
	females	2	2	2	3	2
Love, family, children	males	2	2	4	3	2
	females	3	3	3	2	3
Material values	males	4	4	2	4	4
	females	5	4	5	4	4
Education (career)	males	5	5	7	6	5
	females	4	5	4	5	5
Self-confidence	males	11	11	5	5	7
	females	8	6	6	9	6
Working capacity	males	6	7	10	11	6
	females	11	11	10	7	9
Intellectual development, erudition	males	9	8	6	8	8
	females	6	7	7	6	7
Discipline, honesty, decency	males	12	9	14	12	10
	females	7	10	8	8	8
Physical education and sports	males	8	6	11	13	9
	females	10	14	14	15	14
Independence	males	16	12	8	9	12
	females	9	9	12	11	10
Friends	males	13	10	13	10	11
	females	14	12	11	13	13
Faith in God and spirituality	males	7	14	15	16	13
	females	12	13	13	12	12
Peace of mind	males	14–15	13	12	7	15
	females	13	8	9	10	11
Recognition by others	males	10	16	9	14	14
	females	15	15	15	14	15
Environment protection	males	14–15	15	16	17	16
	females	17	16	16	17	16
Art and culture	males	17	17	17	15	17
	females	16	17	17	16	17

same rating places – the 1st, 2nd, 3rd, 4th in all educational departments. At the same time, physical culture and sports took the 5th place in the values system of the male students of the sports department, and 12th – in the values system of females. In the special education department the physical education and sports was ranked only 12th for males and 15th for females, in the main educational department – 8th and 14th respectively.

DISCUSSION

Providing young people with broad freedoms is not enough for the development of physical culture. We need a socially-oriented cultural policy, scientific knowledge that could form the high aesthetic qualities and positive needs of young people, and help to acquire the necessary means for self-improvement and self-education. Socio-economic factors, environmental issues, the lack of health priority and motivation for a healthy lifestyle as a major factor in health formation, maintenance, preservation, and recovery play an important role. However, prioritizing health, students do not take care of it because there are many self-destructive health practices among young people (smoking, drug abuse, infectious diseases, alcohol abuse, gambling, Internet addiction, game addiction, etc.). It should also be noted that the student environment is unfavorable for the activation of the components of a healthy lifestyle; it promotes the spread of bad habits, the consequences of which students do not realize.

Most students do not have the need to take care of their health developed. Such students rely on their youth, the activities of medical institutions, the authority and professionalism of doctors, and neglect such effective and economically sound means as physical culture and sports. To improve students' health, it is important to engage them in classes of physical activity of person-centered choice during extracurricular activities and to provide the opportunity to continue scheduled physical activity with a sports orientation.

Forming students' health while studying at HEI involves the implementation of a number of areas, namely:

- ensuring real health by increasing the level of adaptive capacity of an organism, the ability to protect (or compensate) from the negative impact or use the positive impact of the factor on health maximally (the prevention of negative effects and the formation of positive ones);
- the creation of a favorable environment by reducing negative conditions or creating the conditions for the maximum realization of the positive properties of the factor (direct focus on health factors);
- ensuring conscious control of one's own health by promoting a healthy lifestyle and valeological education;
- the formation of a system of active health monitoring, which implies a systemic and systematic study of the psychological, physical and somatic components of health and the active use of the acquired information in the process of valeological education [16, 33].

The improvement of the health state and the level of phys-

ical and functional preparedness of students by means of physical education is impossible without taking into account the relevant characteristics of an organism, and obtaining operational information about the students' state through medical and biological methods of research is connected with a number of difficulties. However, the solution to this problem can be facilitated by the results of subjective indicators, which, as a rule, reflect the state of a student's organism and do not require significant material and time costs [19].

The greatest effectiveness of health-improving training can be achieved by using basic methodical principles (gradual and continuous physical activity, the adequacy of physical activity to the level of health, the purposefulness of the means of health-promoting training, rhythmic use of various means), as well as the ratio of time spent on the development of physical qualities: 50-60 % of the total time is dedicated to general endurance, 15-20 % – the strength and strength endurance, up to 10 % – the speed and speed endurance, 5-10 % – the flexibility, 10-15 % – agility [33]. In addition, the authors set the limits of physical activity depending on the level of somatic health of students:

- for a low level: the intensity of classes is 40-50 % of the maximum allowable heart rate; the means include walking, slow running, swimming, strength endurance and flexibility exercises; the frequency of applying in one lesson is 5-7 times per 10-30 minutes; the pace is steady, the priority method is repeated exercises;
- for a middle level: the intensity of classes is 56-66 % of the maximum allowable heart rate; the means include fast walking, slow running, jumping, swimming, strength and speed endurance, agility exercises; the frequency of applying and volume in one session is 5-7 times per 15-40 minutes; the pace is steady, the priority method is circuit training;
- for a high level: the intensity of classes is 70-80 % of the maximum allowable heart rate; the means include exercises for the development of speed-power, power qualities, strength endurance, agility, jumping, swimming; the frequency of applying and volume in one session is 3-5 times per 10-50 min; the pace is steady and variable, the priority method is to perform the exercise to the last extremity.

We agree with the research of A. V. Maglovanyi [34] that the programmatic strategy of physical education should be based on a new paradigm of health, enriched with basic knowledge in the field of biology and physiology of health, physiology of sports, on establishing causative-consecutive relationships between ordinary physical activity, mental capacity and mental health taking into account psychological characteristics of a student's personality. The human body (physical status), one's mind and ability to think (intellectual component), moral and ethical principles (moral component), the ability to control emotions (emotional component), the ability to adapt to the conditions of life and work (a component of social status) are formed and developed. On the basis of the proposed conception, the author distinguishes the components of the health of

a well-developed personality – physical, mental, social statuses; outlines the structure of the constituents of each, which teachers of HEI should take into consideration while working with students, namely the five hierarchically branched levels of health structure. The first level of health structure is integral individual health. The second level is health status, including physical, mental and social. The third level of health structure is formed by the components of statuses. The components of physical health are 1) internal physiological systems of an organism; 2) control systems of an organism. The constituents of mental health are 1) intelligence; 2) emotions; 3) character. The components of social health include 1) adaptation to physical conditions of life; 2) adaptation to working conditions; 3) adaptation to the moral and ethical standards of the environment. The fourth level is the integral components. Internal physiological systems of an organism are 1) physiological systems of the internal sphere (circulatory system, respiratory system, exchange systems); 2) control systems of the body (nervous and endocrine systems). The components of intelligence include 1) logical, associative, algorithmic, spatial thinking; 2) memory; 3) attention; 4) focus; 5) concentration, etc. The constituents of the emotional component embrace 1) mental experiences – the internal (impressive) mental constituent of emotions; 2) the display of emotions externally (motor and behavioral acts, facial expressions, speech) – the external (expressive) mental constituent of emotions. The constituents of the characterological component are the qualities of a person that are responsible for preserving and maintaining health (for example, optimism, purposefulness, etc.), that is, the system of character traits that determine the degree of self-organization and self-education of an individual. The integral components of adaptation to the physical conditions of life and social status include 1) the claims on the degree of comfort; 2) mobility; 3) physical abilities of adaptation to environmental conditions. The constituents of adaptation to working conditions embrace 1) adaptation to professional requirements; 2) adaptation to the intellectual surrounding of the professional environment; 3) the ability to overcome psychological barriers. The integral components of adaptation to the moral and ethical standards of the environment are 1) psychological compatibility with family environment; 2) communicativeness; 3) interpersonal relationships. The fifth level of health structure is formed by 1) the indicators of physiological systems of an organism, measured by laboratory or clinical diagnostic methods or obtained by calculations; 2) mental and social health indicators, determined by test and survey methods [21, 33, 34].

The development of new scientifically sound ways of engaging students in physical education requires several methods to solve this problem, the main of which is the development of ways to increase students' physical activity, that is, engaging young students in independent exercises and sports in their spare time, combating bad habits, compliance with nutrition prescription and hygienically sound training regime, work and rest.

The other direction of improving the health of students is the inclusion in the programs of physical education the issues of environmental education that provide students with a system of knowledge about the negative and positive impact of environmental factors on human health, the rules of life in a polluted environment, taking into account the areas of radioactive contamination.

The primary objective of further research is to engage students in the personal-oriented choice of fitness technologies, the development of differentiated methods of physical education classes, the complexes of special physical exercises that will allow increasing motor activity, working capacity, to improve the functional state of an organism, and to preserve students' health while studying at HEI. At the same time, we should undertake extensive educational and explanatory work on:

- the concept of health and its components;
- teaching the methods of determining the physical state and assessing one's own health;
- drawing students' attention to the preservation of their own health as the highest value of life;
- fostering a sense of responsibility for one's own behavior and abandoning self-destructive youth health habits;
- teaching to acquire self-care skills;
- identifying and using the most important priorities of a healthy lifestyle as the factors for forming, preserving, strengthening, restoring and passing on health to the next generations;
- providing students with rational means and methods of physical education for the prevention of cardiovascular diseases, the diseases of the respiratory system, musculoskeletal system, metabolic disorders, etc.;
- the ability to define rational motor activity as a factor of improving one's own health and following it in practice;
- the ability to properly use different types of becoming tempered (sunbathing, air baths, water procedures, winter swimming, walking barefoot, use of baths, etc.);
- the formation of a positive concept of active rest, providing knowledge, skills, and abilities to properly organize one's leisure and the leisure of close people;
- the ability to find one's social status, harmony with nature and one's own place in society.

Thus, the conducted research revealed the following tendency: 1) the deterioration of health state of Ukrainian students; 2) students are diverse for reasons of health; 3) the disease incidence rate of students from the same rural or urban area is dissimilar in different HEI; 4) there are differences in the health state of students from different regions; 5) targeted steps to preserve students' health and improve their lives have a positive tendency to reduce the disease incidence among youth. The results of our studies confirm the conclusions of many scientists [1, 2, 5, 8 and others] and supplement them.

CONCLUSIONS

1. The basis of improving health and increasing the level of physical fitness of students is physical education, aimed at improving functional abilities of an organism,

the development of physical qualities (strength, speed, endurance, flexibility, coordination), simultaneously improving special physical and technical readiness concerning the types of physical activity and acquisition of professionally-applied psychophysical skills and abilities, and in general, aimed at the improvement of health state.

2. The introduction of new forms and technologies of physical education of students, aimed at providing the foundation of health, requires the creation of a fundamentally new infrastructure of the national system of physical education of students. The theoretical, methodical and legal principles of designing this infrastructure and its further exploitation should be laid down in the national doctrine of physical education of the school and university students of Ukraine. The new forms and technologies of physical education of students are ultimately determined not only by the efficiency of using the methods, the availability of training bases, the material and technical support, the initial level of physical fitness and the state of health of students but by the whole complex of environmental, socio-economic, psychological and pedagogical, and sanitary and hygiene factors.

The prospects for further research are to explore the use of the means of health and fitness activities to improve the health of students.

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ORIGINAL ARTICLE

THE MEDITERRANEAN DIET IN THE PREVENTION OF CARDIOVASCULAR AND NERVOUS SYSTEM DISEASES – THE ASSESSMENT OF NUTRITIONAL KNOWLEDGE

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ABSTRACT**Introduction:** Following a Mediterranean diet significantly reduces the risk of cardiovascular and neurodegenerative diseases.**The aim** of the study was to assess nutritional knowledge among adult residents of the Silesian Province on the Mediterranean diet in the prevention of cardiovascular and nervous system diseases.**Material and methods:** The study was conducted using an anonymous self-made questionnaire. 190 adults from the Silesian Province participated in the study.**Results:** The respondents had a good or sufficient level of knowledge on the Mediterranean diet. Respondents as the benefits of using the Mediterranean diet more often indicated a reduction in the risk of cardiovascular diseases than prevention of nervous system diseases.**Conclusions:** The inhabitants of Silesian Province have a fairly good level of knowledge on the preventive use of the Mediterranean diet. Public knowledge on the prevention of cardiovascular diseases, as well as knowledge on the principles of the Mediterranean diet, and on the benefits of its use, can reduce the incidence of cardiovascular and nervous system diseases.**KEY WORDS:** Mediterranean diet, prevention, cardiovascular diseases, nervous system diseases

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INTRODUCTION

A proper diet is one of the very important factors in the prevention of diseases of the nervous and cardiovascular systems [1-7]. The Mediterranean diet is often recommended. The traditional Mediterranean diet is mainly based on the consumption of fresh vegetables and fruits, whole grain cereals, vegetable oils, mainly olive oil, fish and legume seeds. Restrictions include red meat, dairy products, saturated fatty acids, and alcohol [8].

Following a Mediterranean diet can significantly reduce the risk of cardiovascular disease, including stroke and heart attack [7]. The beneficial effects of the Mediterranean diet are probably related to the action of the nutrients it contains, which may have an effect on lowering blood pressure, lowering lipids, improving metabolism and preventing inflammation in the body [7-11].

It was also shown that following a Mediterranean diet may have a beneficial effect on reducing the risk of neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease [6, 12]. The following nutrients supplied to the body during the diet are responsible for beneficial effects on the nervous system: antioxidants (vitamin C, vitamin E), mono- and polyunsaturated fatty acids, coenzyme Q10, glutathione, flavonoids, curcumin, and caffeine [2].

THE AIM

The aim of the study was to assess nutritional knowledge among adult residents of the Silesian Province on the use of the Mediterranean diet in the prevention of cardiovascular and nervous system diseases.

MATERIAL AND METHODS

190 inhabitants of the Silesian Province, 125 women (65.8%) and 65 men (34.0%) participated in the study.

The research tool was the author's proprietary anonymous questionnaire, consisting of single and multiple choice questions and open questions. One point was given for each correct answer, and 0 – for every incorrect answer and “I don't know” answer. The maximum number of points was 26. In order to assess the knowledge of the respondents, the following point scale was established: 0 – 7 points – unsatisfactory (E); 8 – 13 – admitting (D); 14 – 18 – satisfactory (C); 19 – 23 – good (B); and 24 – 26 – very good (A).

RESULTS

The largest part of the study group were people aged 18 – 29 (n = 145; 76.3%). 148 people (77.9%) lived in the

Table 1. General knowledge on the Mediterranean diet.

Question	Possible answers	n [%]
Definition of the Mediterranean diet	Nutritional habits of people living in the Mediterranean*	114 [60.0%]
	Type of slimming diet	13 [6.8%]
	Diet based on the complete elimination of meat from the diet	13 [6.8%]
	Diet based mainly on eating vegetables and fruits	50 [26.4%]
Benefits of using the diet	Reduction of cardiovascular risk *	175 [92.1%]
	Reduction of the risk of cancer *	84 [44.2%]
	Prevention of peptic ulcer disease	28 [14.7%]
	Slowing down the aging process of the nervous system *	79 [41.6%]
Diseases which risk can be reduced by following a Mediterranean diet	Myocardial infarction*	171 [90.0%]
	Ischemic heart disease*	142 [74.7%]
	Stroke*	94 [49.5%]
	Celiac disease	2 [1.1%]
	Alzheimer's disease*	70 [36.8%]
	Cancer*	82 [43.2%]
	Osteoporosis*	29 [15.3%]
Reducing the risk of developing cardiovascular disease	Peptic ulcer disease	34 [17.9%]
	Yes*	161 [84.7%]
	No	3 [1.6%]
	I don't know	26 [13.7%]
Influence on life length	Yes*	153 [80.5%]
	No	5 [2.6%]
	I don't know	32 [16.9%]
Use in obesity prevention	Yes*	140 [73.7%]
	No	6 [3.3%]
	I don't know	44 [23.2%]
Decrease the risk of neurodegenerative diseases	Yes*	95 [50.0%]
	No	15 [7.9%]
	I don't know	80 [42.1%]
Effect on total serum cholesterol level	Decrease the level*	138 [72.6%]
	Increase the level	1 [0.5%]
	No influence on the level	9 [4.8%]
	I don't know	42 [22.1%]

* correct answers

city, while 42 people (22.1%) lived in a rural area. The majority of respondents (n = 118; 62.11%) had secondary education.

The vast majority of respondents (n = 178; 93.7%) did not report diagnosed cardiovascular and nervous system diseases. Other respondents indicated hypertension, varicose veins of the lower extremities and atrial fibrillation. In families of respondents the following diseases were present: hypertension (n = 96; 50.5%), prior myocardial infarction (n = 78; 41.1%), stroke (n = 45; 23.7%), ischemic heart disease (n = 20; 10.5%), dementia (n = 30; 15.8%), Parkinson's disease (n = 13; 6.8%), venous thrombosis (n = 30; 15.8%) and atherosclerosis (n = 47; 24.7%).

60% (n = 114) of the respondents knew the correct definition of the Mediterranean diet. The most common benefits of the Mediterranean diet were associated with a reduced risk of cardiovascular diseases. To a lesser extent, the respondents believed that the Mediterranean diet would have an impact on reducing the risk of developing cancer or nervous system diseases. The vast majority of respondents (n = 153; 80.5%) thought that life expectancy was to some extent dependent on the use of the Mediterranean diet. About 70% of respondents (n = 140) associated the Mediterranean diet with obesity prevention. In addition, approximately 70% (n = 138) were of the opinion that total serum cholesterol levels would decrease due to the Mediterranean diet (Table 1).

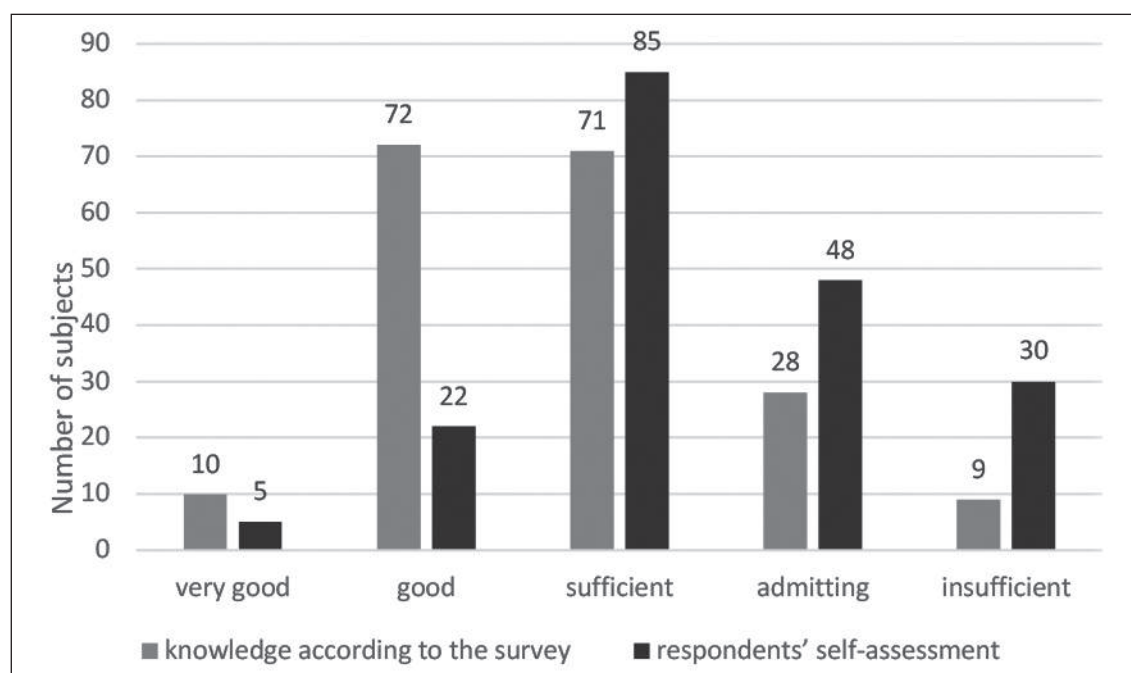


Fig. 1. The level of respondents knowledge according to survey results and self-assessment – the comparison.

The main source of fat in the Mediterranean diet is olive oil. Almost 90% ($n = 170$) of the respondents had correct knowledge on this subject. About 60% ($n = 113$) found that the recommended amount of vegetables and fruits in the Mediterranean diet is 4-5 servings. Most respondents knew that fish consumption should be at least twice a week ($n = 151$; 79.5%). In the question about red wine, more than half of the respondents ($n = 108$; 56.8%) chose the correct amount of alcohol. Fish as the product that should be most often found in the Mediterranean diet was indicated by 173 respondents (91.1%). In the question about fatty acids in which the Mediterranean diet is rich, half of the respondents chose all three correct answers. Most often, the Mediterranean diet was associated with omega-3 ($n = 160$; 84.2%) and / or omega-6 ($n = 89$; 46.8%) fatty acids. Most of the respondents ($n = 143$; 75.3%) indicated vegetable protein as the main source of protein. Most often, the respondents ($n = 165$; 86.8%) gave the correct answer that dark bread should predominate in the Mediterranean diet (Table 2).

The vast majority of respondents ($n = 165$; 86.8%) were aware that in addition to the Mediterranean diet, physical activity also played an important role in the prevention of cardiovascular and nervous system diseases. 25 people (13.2%) believed that physical activity was not significant or did not have knowledge about it.

The results of the survey indicate that the respondents most often presented a good or sufficient level of knowledge. A comparison of the results obtained with the results of the respondents' self-assessment showed that the self-assessment of the respondents who obtained a very good or good grade was lower than their actual results, while the self-assessment of people whose knowledge was assessed as sufficient, admitting or insufficient was higher. (Fig. 1).

DISCUSSION

In recent years an increase in the incidence of cardiovascular and nervous system diseases has been observed. In the management of these diseases, prevention is extremely important, among others, by changing incorrect eating habits and adequate physical activity. In the prevention of many diseases of the nervous system, as well as cardiovascular system, the Mediterranean diet has proven effective, which is considered one of the healthiest diets in the world [3].

In this study, the majority of respondents (84.7%) knew that the Mediterranean diet reduced the risk of developing cardiovascular diseases. These results were similar to those reported in the available literature. In 2017, a survey was conducted among 200 students of nursing, medicine, emergency medicine and obstetrics studying at the Medical University of Lublin. 74.5% of students were of the opinion that "the Mediterranean diet consisting of eating whole grains, unprocessed meat, seeds, nuts, vegetables, unsaturated fats" is a diet recommended for the prevention of cardiovascular diseases. However, 39% of respondents incorrectly indicated that a diet high in polyunsaturated fatty acids is a risk factor for coronary heart disease. The highest level of knowledge about the risk factors of ischemic heart disease was presented by midwifery students, and the lowest – by emergency medical students [14].

The vast majority of respondents were also aware that physical activity plays an important role in the prevention of cardiovascular and nervous system diseases. Similar conclusions were made in the study of Krzyżanowska et al. In 2017, in which 76.5% of respondents showed a high level of knowledge about the impact of physical activity on the risk of cardiovascular diseases [14]. Also, in a study among students of the Medical University of Wrocław, it was shown that 98.9% claimed that physical activity was important in the prevention of cardiovascular diseases [15].

Table 2. Nutritional recommendations in the Mediterranean diet

Question	Possible answers	n [%]
Basic source of fat	Olive oil*	170 [89.5%]
	Butter	1 [0.5%]
	Lard	4 [2.1%]
	Coconut oil	15 [7.9%]
Number of servings of vegetables and fruits	Less than 2	2 [1.0%]
	2 – 3	57 [30.0%]
	4 – 5*	113 [59.5%]
	6 or more	18 [9.5%]
Frequency of fish consumption	Once a week	38 [20.0%]
	At least 2 times per week*	151 [79.5%]
	Once a month	0 [0.0%]
	Less than once a month	1 [0.5%]
Amount of red wine allowed	At least one glass per day	41 [21.6%]
	Maximum one glass a day for women and 2 glasses a day for men*	108 [56.8%]
	Maximum one glass a month	22 [11.6%]
	Prohibited in the Mediterranean diet	19 [10.0%]
Food product that should be consumed most often	Red meat	3 [1.5%]
	Fish*	173 [91.1%]
	White meat	7 [3.7%]
	Eggs	7 [3.7%]
Fatty acids in which the Mediterranean diet is rich	Monounsaturated fatty acids*	48 [25.3%]
	Saturated fatty acids	28 [14.7%]
	Fatty acids omega-3*	160 [84.2%]
	Fatty acids omega-6*	89 [46.8%]
Protein predominating in the Mediterranean diet	Vegetable Protein*	143 [75.3%]
	Animal Protein	47 [24.7%]
Type of bread	Light	25 [13.2%]
	Dark*	165 [86.8%]

* Correct answer

The subjects were also asked about the role of the Mediterranean diet in maintaining a healthy body weight. Most of the respondents knew that this diet could be used to prevent obesity. This diet turns out to be effective not only in the prevention of obesity, but also in its treatment. In the study of Rajewski et al. concerning obese women, a diet based on the principles of the Mediterranean diet was used and appropriate physical activity was performed. The results of this study confirmed the effectiveness of this diet and adequate physical activity in the treatment of obesity [16]. Also, in the study of Schlegel-Zawadzka et al. comparing the diet of adult residents of Poland and Greece, it was shown that obesity was much less common in people using the recommendations of the Mediterranean diet [17].

The author's survey also asked about the amount of red wine allowed during the day according to the recommenda-

tions of the Mediterranean diet. The correct answer, which was “a maximum of one glass a day for women and two glasses a day for men”, was given by more than half of the respondents. Similar results were obtained by Krzyżanowska et al. The majority of students (59.0%) answered correctly that in cardiovascular diseases the maximum permissible amount of ethyl alcohol is 20-30 g for men and 10-20 g for women [14].

During 25 years of observation of the diet of randomly selected populations, a relationship was found between saturated fatty acid intake and mortality due to coronary heart disease. The inhabitants of Japan, whose diet contained the least saturated fatty acids, were also characterized by the lowest mortality rate due to coronary heart disease. This rate was about six times lower compared to the population of Eastern Finland where the consumption of saturated fatty acids was the highest [18].

In the author's study, a percentage of 25.3% of respondents believed that the Mediterranean diet was rich in monounsaturated fatty acids. Especially monounsaturated fatty acids were used in the prevention of cardiovascular diseases [19, 20]. At the same time, it was confirmed that the consumption of excessive amounts of saturated fatty acids was synonymous with an increased risk of coronary heart disease [20].

In this study, the knowledge of the inhabitants of the Silesian Province was mostly classified at a good or sufficient level. The respondents had information on the type of fat used in the Mediterranean diet, the frequency of consumption of fish, vegetables and fruit, and the type of bread recommended. However, knowledge does not always translate into its practical application. In one of the available studies, conducted among 151 students of medicine and dietetics (aged 21 to 23) of the Medical University of Warsaw, the correctness of their diet with the principles of the Mediterranean diet was assessed. The diet in this group was assessed by the method of eating food frequency in the 3 months preceding the study. Then, data averaged over the week period was calculated using the value of the Mediterranean diet compliance index. It is the ratio of energy that comes from products characteristic of the Mediterranean diet to energy from other consumed products. This indicator was adapted to Polish conditions. Researchers drew the basic conclusion that the diet of the studied group of students was significantly different from the principles of the Mediterranean diet. Despite the declaration of the Mediterranean diet by several medical students, the calculated index was higher than the average of the other students, however it was much lower than the index determined for the Mediterranean diet. Also, the amount of food consumed in this group was not fully in line with the principles of the Mediterranean diet. Therefore, all students should change their current diet model by increasing the consumption of fruit, pulses and vegetable fats, and reduce the consumption of animal fats [21].

The Mediterranean diet finds its application in the prevention and treatment of cardiovascular diseases. In one publication, researchers attempted to assess the effectiveness of information on the principles of proper nutrition, especially the principles of the Mediterranean diet, provided by the attending physicians. The target group was 78 patients aged 40–82, treated in Warsaw cardiology clinics. It was shown that these patients did not have sufficient knowledge on the principles of nutrition. However, most of them were willing to receive additional information. Only 12.0% of patients received a recommendation to implement a Mediterranean diet from a doctor. The same percentage (12.0%) was encouraged to take advantage of the dietary advice [22].

Public knowledge on the prevention of cardiovascular diseases, as well as knowledge on the principles of the Mediterranean diet, as well as the benefits of its use can reduce the incidence of cardiovascular and nervous system diseases.

CONCLUSIONS

Inhabitants of the Silesian Province have a sufficient level of knowledge about the preventive use of the Mediterranean diet. Respondents as the benefits of using the Mediterranean diet more often indicated a reduction in the risk of cardiovascular disease than in the prevention of nervous system diseases.

Public knowledge on the prevention of cardiovascular diseases, as well as knowledge on the principles of the Mediterranean diet, as well as the benefits of its use, can reduce the incidence of cardiovascular and nervous system diseases.

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Conflict of interest

Authors declare no conflict of interest.

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ORIGINAL ARTICLE

THERAPEUTIC PLASMAPHERESIS IN A COMPLEX TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS

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ABSTRACT

The aim: To determine the pathogenetic expediency, efficiency and the place of therapeutic plasmapheresis in a complex treatment of patients with chronic hepatitis.

Materials and methods: It was carried out the analysis of case histories of 77 patients. In the course of treatment, the patients were diagnosed with chronic toxic hepatitis (K.71). Diagnosis was exposed in accordance with the official documentation introduced by the Gastroenterological department of Sumy Regional Clinical Hospital CH "SRCH", Sumy Regional Infectious Diseases Clinical Hospital named after Krasovitsky ZY and Sumy Regional Center of Blood Service.

Results: It was found that total protein indicator is the normal range and albumin after plasmapheresis and during conservative treatment.

Markers of cytolysis and cholestasis are have great value may. The most significant changes were observed in rates indicating lesion of the hepatic parenchyma, including ALT, AsAT and alkaline phosphatase. In conservative treatment, the percentage of the alkaline phosphatase level improved by 31%, after the course of plasmapheresis – by 58%. The obtained figures of cholestasis indexes indicate the effectiveness of both methods of treatment, but treatment with plasmapheresis has a more expressed effect on the decrease of alkaline phosphatase level. The level of bilirubin improve on 38,8% after plasmapheresis and 65% in the comparison group.

Conclusion: Inclusion in the complex therapy of chronic hepatitis plasmapheresis reduces the severity of cytolysis and cholestasis, increases remission and significantly improves clinical manifestations.

KEY WORDS: hepatitis, plasmaferesis, remission

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INTRODUCTION

Chronic diffuse liver diseases (CDLD) remain one of the most urgent problems of the present, as they were before, due to their widespread prevalence, pathogenetic mechanisms, which are not fully studied, unresolved problems of treatment, prevention and unfavorable prognosis. The majority of liver diseases treated by therapists are chronic hepatitis. This is a group of diseases caused by various reasons, characterized by focal hepatocellular necrosis of varying duration and chronic inflammation. The concept of "chronic hepatitis" is determined by the disease duration, the conditional limit is 6 months [1, 2]. Four types of chronic hepatitis are distinguished: viral, autoimmune, drug-induced and cryptogenic. For today, hepatologists identify the fifth type of the disease as alcoholic chronic hepatitis [3,4]. Chronic viral hepatitis is more often caused by hepatitis B (HBV), C (HCV) and D (HDV) viruses, the last one is usually coincided with hepatitis B. High infection rate with the HBV virus of the planet's population is related to its extraordinary prevalence rate and resistance, which is 50-100 times higher than that for HIV [5]. An important place among hepatitis is drug-induced, caused by hepatotoxic drugs [6].

Medicinal liver damage is reason for approximately 2% of liver jaundices. At present, there are known more than 1,000 drugs that can cause drug-induced hepatitis. Liver lesions are more common in enteral use of drugs, due to the peculiarities of their metabolism and blood supply of hepatic tissue [7,8].

Depending on the influence of the etiological factor, the synthetic activity of the liver is reduced, the detoxication function suffers and the regulation of metabolic processes is violated. The imbalance between the physiological needs and the functional capabilities of the damaged liver leads to the development of complications [9].

In this regard, the problem of finding additional therapeutic measures, including the development and introduction of new methods of pathogenetic therapy remains relevant. There are publications about the successful use of the apheresis method in modern literature. The following types of apheresis are distinguished: sorption, cypruss, high-volume/massive plasma exchange (HVP), immunosorbition (IS), apheresis LDL, plasmapheresis. Plasmapheresis is a method of efferent therapy in which the patient or donor's blood is passed through a medical device that separates the plasma from other components of the blood. Plasma is

removed and replaced by a plasma replacement solution (for example: albumin, NaCl 0.9%, donor plasma) [10]. It is important the type of solution that should be used in the process of therapeutic apheresis for a practitioner, as well as the state of the factors of blood coagulation, since there is a risk of exhaustion of coagulation factors, especially after weekly PF and the timing of the procedure. There are two main methods of automatic plasmapheresis: centrifugal and filtration. The first method is carried out by centrifugation of blood with a constant or intermittent flow of it in special devices, the other one is based on blood filtration in special plasma filters [11].

THE AIM

The aim of our research is to determine the pathogenetic expediency, efficiency and place of therapeutic plasmapheresis in the complex treatment of patients with chronic hepatitis.

Objectives of the study: 1. To establish the validity of conservative treatment effect and treatment effect with the addition of plasmapheresis on hematological parameters after 2 weeks.

2. To determine the economic effect of the inclusion of plasmapheresis in therapy.

MATERIALS AND METHODS

The research was conducted by the Department of Public Health in the Medical Institute of Sumy State University. The analysis of the case histories was carried out on 77 patients (43 men and 34 women) aged from 15 to 75 years. In the process of treatment, patients were diagnosed with chronic toxic hepatitis (K.71). Diagnosis of patients was exhibited according to the official documentation by the implemented Gastroenterological department of Sumy Regional Clinical Hospital "SRCH", Sumy Regional Infectious Diseases Clinical Hospital named by Krasovyt'skyi Z.Y. and Sumy Regional Blood Service Center according to the ICD-10.

All patients were divided into two representative groups with the only conservative drug therapy ($n = 39$) and conservative treatment with therapeutic plasmapheresis ($n = 38$).

A conservative therapy of hepatitis included a diet limitation of protein in the food; correction of hemostasis, hypoalbuminemia and water electrolyte state; infusion-detoxification therapy; prevention of erosive-ulcerous lesions of the gastrointestinal tract; conducting a medical therapy aimed at reducing liver encephalopathy.

In this case, a device for membrane plasmapheresis AMPA-TT (trademark "Hemofenix") was used, with the complex of a plasma filter PFM-TT "ROSA" and blood-flow lines. The process of membrane plasmapheresis on the device "Hemofenix" is carried out according to a single-needle scheme in a sterile extracorporeal contour of a single use. Vascular access was performed through peripheral subcutaneous veins (elbow vein). Average

duration of the procedure is $1\text{-hour} \pm 10\text{minutes}$. During the procedure, an average of 650 ± 30 ml of plasma was obtained. During the operation, the patient was given NaCl 0.9% – 200 ml, rheosorbilact – 200 ml, glucagon – 250 ml, heparin – 10% OD. In some patients instead of sorbilact/rheosorbilact were used rheopolyglucinum – 200 ml or refortanum – 200 ml. In total, for treatment, there were received an average of 3370 ml. of plasma (about 1 volume of circulating blood). The number of procedures for therapeutic plasmapheresis, the maximum number of performed apheresis is eight, the minimum are 3 times at intervals between procedures for 2 days. To control the parameters of homeostasis, a complex of clinical and instrumental-laboratory methods was used. Among the clinical methods, the following parameters were assessed: general condition, skin color, blood pressure, pulse and body temperature, which were performed for all patients before, during and after the method of extracorporeal hemocorrection. Among instrumental and laboratory methods: mesenchymal inflammation (total protein, albumin), cytotoxic markers (ALT, AsAT) and cholestasis (activity of alkaline phosphatase, bilirubin) were studied. Blood samples for laboratory control of the studied parameters were taken during the admission of the patient in the hospital (before treatment) during a conservative therapy and after the application of the course of therapeutic plasmapheresis.

Albumin index was determined by electrophoresis on cellulose acetate tapes.

Alanine aminotransferase (ALT) was determined by the kinetic method of the automatic analyzer Sobas C311 using the test system: AST, Roche Diagnostics.

Aspartate aminotransferase (AsAT) was determined by the kinetic method of the automatic analyzer Sobas C311 using the test system: AST, Roche Diagnostics.

Rates of alkaline phosphatase were calculated by the colorimetric method of the Sobas C311 analyzer using the test system: ALP2 Roche Diagnostics.

The level of total bilirubin was determined by the Endrass-Klegorn-Grof method.

The study was conducted in accordance with the requirements of medical statistics, on the condition of the informed voluntary consent of patients for the processing of personal data. The resulting digital information was processed with using Microsoft Excel 2010, Statistica 10 (StatSoft Inc. USA) software using descriptive, comparative analysis.

The general statistical analysis of determining the authenticity of the difference in quantitative characteristics between the samples was used by Student's criterion. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

According to the results of the monitoring of patients with CH, changes in clinical and laboratory parameters were established. After analyzing 77 stories of the disease, the following distribution of patients was found: 34 (44%) women and 43 (56%) men.

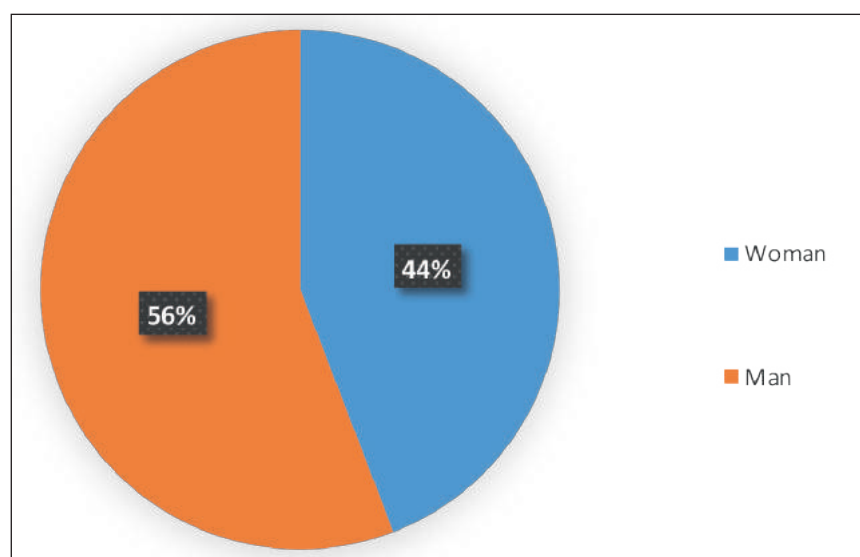


Fig 1. Sexual distribution of persons

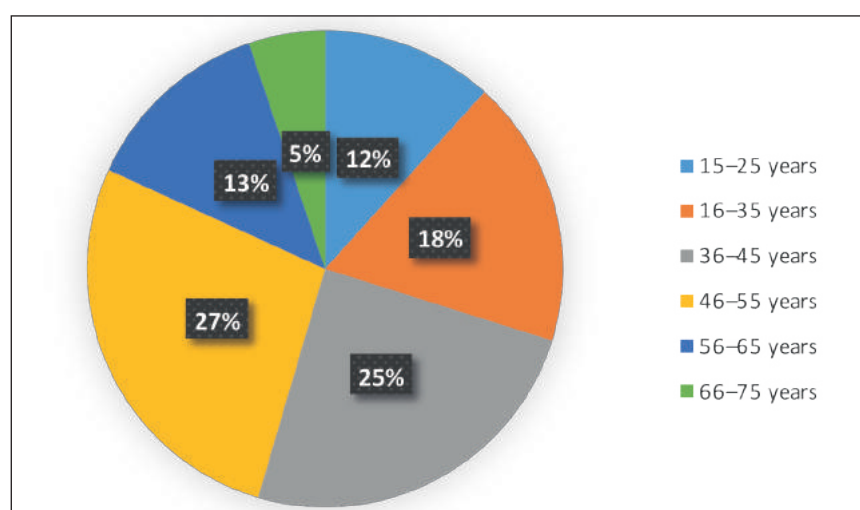


Fig 2. Age distribution of patient

According to the age, all patients were divided into the following groups: from 15 to 25 years old – 9 (12%), from 26 to 35 years old – 14 (18%), from 36 to 45 years old – 19 (25%), from 46 to 55 years old – 21 (27%), from 56 to 65 years old – 10 (13%), from 66 to 75 years old – 4 (5%).

According to the results of the monitoring patients, there were diagnosed the leading clinical syndromes, the most marked were astenovegetative and dyspeptic. Also among patients were found hepatomegaly (enlargement of the liver for 6-8cm), yellowness of the skin and itchy skin. By evaluating the laboratory rates, changes in mesenchymal inflammation, markers of cytolysis and cholestasis were determined by us.

A rank evaluation of the degree of accuracy (according to the t-criterion) of all studied rates of homeostasis in the patients under the influence of treatment plasmapheresis and a conservative treatment showed that membrane plasmapheresis with the conservative method, had a more marked effect on homeostatic functions. These studies in groups are presented in Table I.

Analysis of the data obtained during the work showed that comparing the results of biochemical studies in

classical therapy and therapy using plasmapheresis, the greatest effect was observed precisely when using plasmapheresis.

In the course of scientific work, were determined changes biochemical parameters. It was found that the level of total protein on the background of the plasmapheresis procedure (before the procedure – $73,6 \pm 5,2$ g/l ($p < 0,05$), after – $68,9 \pm 5,1$ g/l ($p < 0,05$)) and during conservative treatment (before treatment initiation – $76,4 \pm 8,8$ g/l ($p < 0,05$), after treatment – $72,4 \pm 6,1$ g/l ($p < 0,05$)) has not changed significantly and is within the normal range, indicating the same effect of conservative and plasmapheresis treatment on this indicator. It should be noted that in the first day was determined a significant decrease in total protein after plasmapheresis, but after 2 weeks the figure returned to normal.

Albumin after 2 weeks of treatment did not change statistically, this indicates that the effect of plasmapheresis on this indicator during the specified period is unreliable.

The most significant changes were observed in rates indicating lesion of the hepatic parenchyma, including ALT, AsAT and alkaline phosphatase. During a conservative

Table I. Dynamics of homeostasis rates in patients with CH under a conservative treatment and using plasmapheresis.

Rates	Norm	Before treatment	After treatment	Reliability of influence
Conservative treatment				
Total protein, g/l	65-85	76.4±8.8*	72.4±6.1*	–
Albumin, %	36-50	43.1±2.5*	42.9±1.0*	–
AsAT, O/L	10-30	90.7±20.1*	40.1±15.1*	+
ALT, O/L	7-40	76.7±20.2*	40.2±12.0*	–
Alkaline phosphatase, O/L	39-117	152.0±19.1*	105.5±20.3*	–
Total bilirubin, mcmol/L	3,4-20,5	67.2±10.0*	41.1±8.1*	+
Treatment with plasmapheresis				
Total protein, g/l	65-85	73.6±5.2*	68.9±5.1*	–
Albumin, %	36-50	45.7±2.3*	43.8±3.1*	–
AsAT, O/L	10-30	125.6±41.2*	37.1±14.2*	+
ALT, O/L	7-40	94.2±23.4*	38.3±15.1*	+
Alkaline phosphatase, O/L	39-117	174.9±41.8*	73.1±27.4*	+
Total bilirubin, mcmol/L	3,4-20,5	74.1±21.4*	25.5±8.1*	+

*the indicator is considered unreliable $p < 0.05$

treatment, the AsAT rates were: till $90,7 \pm 20,1$ O/L against $40,1 \pm 15,1$ O/L, in the percentage the indicator changed by 29%. During therapeutic plasmapheresis: before the procedure was $125,6 \pm 41,2$ O/L, against $37,1 \pm 14,2$ O/L, the percentage changed by 70%.

Indicators of ALT to a conservative treatment were $76,7 \pm 20,2$ O/L, against $40,2 \pm 12,0$ O/L, the normalization of the indicator is 48%. In the course of therapeutic plasmapheresis: till $94,2 \pm 23,4$ O/L, against $38,3 \pm 15,1$ O/L the rate changed by 59%. The obtained figures points out the effectiveness of both methods of treatment, but plasmapheresis has more expressed effect on the decrease of cytotoxic markers.

Having evaluated the level of alkaline phosphatase in the main group and the comparison one, it was before the procedure 152.0 ± 19.1 O/L, after 105.5 ± 20.3 O/L, in contrast to the control group (before 174.9 ± 41.8 O/L, after was 73.1 ± 27.4 O/L). In conservative treatment, the percentage improved by 31%, after the course of plasmapheresis – by 58%.

Further research of cholestasis indexes points out changes in the level of total bilirubin. Normally, it is 3.4-20.5 mcmol/L. There is a deviation from these normative figures in both groups. In the main group before of plasmapheresis procedures, it is 74.1 ± 21.4 mcmol/L, against 25.5 ± 8.1 mcmol/L, the index has changed by 66%. In the comparison group: before is 67.2 ± 10.0 mcmol/L, after – 41.1 ± 8.1 mcmol/L, the improvement is by 39%. The obtained figures indicate the effectiveness of both methods of treatment, but treatment with plasmapheresis has a more expressed effect on the decrease of alkaline phosphatase level.

DISCUSSION

The analysis of the database showed that the conservative therapy led to the improvement in the patient's condition less than after plasmapheresis. The synthetic function of the liver was recollected more slowly. The level of total protein, albumin remained within the norm in 2 weeks.

Patients in the group with therapeutic plasmapheresis were performed, showed the improvement in the general condition: faster asteno-vegetative and dyspeptic syndromes, decreasing of skin itching and intoxication symptoms. After courses of plasmapheresis at intervals of 2 days, there was a significant decrease in AsAT, ALT, AP and total bilirubin, which was maintained 2 weeks after the completion of the procedures. Due to the adequate replacement of single-group plasma losses, the patients are registered with the improvement in albumin and total protein rates, although they were also within normal parameters. Thus, complex treatment of CH with the inclusion of plasmapheresis has a positive effect on the disease due to the mechanical removal of toxic products from the blood of patients.

The statistical data processing demonstrates a more valid treatment effect on hematological parameters when plasmapheresis is included in the course of therapy. Conservative therapy showed statistically significant effects on AsAT and total bilirubin.

Therapy with plasmapheresis showed statistically significant effects on AsAT, ALT, alkaline phosphatase and total bilirubin. Thus, it is the greater the therapy effectiveness with the inclusion of plasmapheresis.

To study the prolonged action of plasmapheresis, we conducted an analysed repeated referrals of patients. In conservative treatment, the average interval between appeals was 18 months and 22 months after plasmapheresis therapy.

As a result of a survey of patients who were discharged at the time of publication after an in-hospital treatment, the average cost of therapy was 42.5 euros. According to a sample retrospective study of a history of 10 years, the cost of therapy for 1 patient at treatment every 18 months (7 times for 10 years) is $42.5 \times 7 = 297.5$ euros.

The cost of the plasmapheresis procedure on Scinomed machine we have taken to calculate is 19 euros (basic fixed and variable costs, including staff salaries and utilities according Ukrainian law, are also taken into account).

The course of treatment consists of 3 procedures as minimum, which require 57 euros. For 10 years the cost of treatment with plasmapheresis for 1 patient at treatment every 22 months (5 times for 10 years) is $57 \times 5 = 285$ euros.

Today the cost of therapy is 297.5 euros, the economic effect during 10 years from the implementation of plasmapheresis can be estimated at $297.5 - 285 = 12$ euros for each patient.

Therefore, the economic effect for Ukraine can be calculated as follows.

According statistics there are 104879 cases with such a pathology are officially registered in Ukraine, then the inclusion of plasmapheresis can have an economic effect for the year $12 \times 104879 = 209758$ euros.

And considering that only 7% of the estimated number of patients is recorded, then the possible economic effect is: $12 \times 104879 \times 100 / 7 = 2\,996\,542$ euros

These data indicate the need for further more in-depth study of ways to optimize the treatment of nosologies, in which the effectiveness of plasmapheresis is proven.

CONCLUSIONS

The absence of a significant and stable effect of the conservative treatment of CH indicates the need to improve the quality of a medical care for patients with CH by using more effective up-to-date techniques, such as usage of plasmapheresis in a complex of therapeutic measures. Inclusion plasmapheresis in the complex therapy of CH reduces the express of cytolysis and cholestasis, increases remission and significantly improves clinical manifestations. Therefore, it is relevant to use this technique in clinical practice.

It is determined that the inclusion of therapeutic plasmapheresis in the complex therapy of hepatitis leads to an economic effect and requires further study of the impact of this method with a view to its implementation through the use of modern economic and marketing methods not only at the state level, but also in the private family medicine sector.

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ORIGINAL ARTICLE

DERMAL ABSORPTION OF DIQUAT AND POTENTIAL OCCUPATIONAL RISK

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ABSTRACT

The aim: The toxicological-hygienic assessment of dermal absorption of diquat in terms of potential risk of its bioavailability in professional use.**Materials and methods:** The object of the study was cutaneous exposure of diquat, determined in toxicological experiments of different duration (data of scientific literature) and at the stage of state testing of pesticide preparations based on diquat dibromide (data of a full-scale hygiene experiment, prognostic model of risk assessment), the technical concentrate of diquat dibromide (active substance content not less than 377 g / kg) contains relevant supplements, the content of which is regulated by the Food and Agriculture Organization.**Results and conclusions:** Due to the high risk of the diquat adverse effects affecting the personnel, general public and environment, the European Union has introduced administrative decisions to forbid plant protection products containing the diquat. Fulfillment of the conditions of the Association Agreement between Ukraine and the European Union indicates the need to develop common regulations and risk assessment methods aimed at ensuring high level of protection of human health and the environment.**KEY WORDS:** diquat, dermal absorption, occupational risk

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INTRODUCTION

The research of dermal absorption (absorption through the skin) using standard experimental procedures is a mandatory step in testing of the active substance of the pesticide for its subsequent registration and application in the market of plant protection products [1-3].

Diquat is one of the pesticides with high risk of dangerous effects on health of workers, including dermal route of entry into the body. Acceptable operator exposure level (AOEL) was set at 0.0002 mg / kg bw by the European Food Safety Authority (EFSA), which is an order of magnitude lower than the acceptable daily intake (ADI) for the general public [2].

The AOEL value was justified on the basis of "no observed" NOEL level of 0.5 mg/ kg established in dog experiments while assessing chronic (within 1 year) toxicity of diquat, using an oral absorption factor of 0.04 and a coefficient of 100. This value is consistent with the results obtained in the short-term inhalation experiment in rats, based on the "No observed adverse effect concentration" (NOAEC) of 0.1 µg / l (equivalent to 0.02 mg / kg body weight) and the coefficient stock – 100 [2].

The high probability of negative effect of diquat on personnel penetrating the body through the skin is evidenced by a special mark, which was introduced when approving maximum permissible concentration (MPC) of pesticide in the working area air – 0.05 mg / m³ as «dangerous for skin» [3].

Diquat was synthesized in the 1960s by Syngenta Crop Protection AG (Switzerland). The preparative forms such

as water-soluble concentrates based on diquat dibromide, which are used for desiccation of crops of sunflower, soybean, peas, cereals, rapeseed, potatoes are registered in Ukraine [4].

Diquat is a non-selective contact herbicide and desiccant, belongs to the class of chemical compounds derived from bipyridylium. It is absorbed by leaves of the plants, spreads by the xylem and, due to its phytotoxic properties, leads to rapid drying of all green parts of the plant, thereby accelerating uniform maturation and early harvesting time in all weather conditions [5]. By mechanism or site of action on plants, bipyridylium derivatives are electron acceptors in photosystem 1 (FS1) of chloroplasts, leading to a significant increase in the content of super oxidant radicals and hydrogen peroxide, which, when reacting with each other, form a hydroxyl radical – a potential inducer of peroxidation reaction, factor of damage to membranes and destruction of photosynthetic apparatus [6].

Another representative of this class of chemical compounds is paraquat, which is prohibited for use in Ukraine and Europe due to its high toxicity (LD₅₀ for experimental animals at oral admission is 30-50 mg / kg), environmental resistance, ability to cause pulmonary sclerosis and acute fatal poisoning in humans (lethal dose of paraquat for humans is 3-5 g).

Diquat is less toxic than paraquat, but mortality from poisoning can be as high as 50% [7]. Basically, poisoning is observed in the inhalation and oral routes of penetrating the body. The potential effect of diquat on personnel is

Table I. Acute toxicity of diquat and diquat of dibromide for rats

Toxicometric parameter	Indicator value	
	diquat ion [10]	diquat dibromide [11]
Acute oral toxicity, LD ₅₀ *, mg / kg	214 (♂), 222 (♀)**	1009 (♂), 1047 (♀)
Acute dermal toxicity, LD ₅₀ *, mg / kg	> 424 (♂;♀)	> 2000 (♂;♀)
Acute inhalation toxicity, LC ₅₀ *, mg / l	0,121 (♂); 0,132 (♀)	0,8 (♂); 1,09 (♀)

Note: * LD50 (LC50) – median lethal dose (concentration); ** ♂ / ♀ – males / females, respectively

Table II. In vitro dermal absorption of diquat in human and in rat

Dilution, mg / ml	Application rate, mg / cm ²	Dermal absorption rate, mcg / cm ² / year	Absorbed dose, % (in 24 h)
<i>Human skin</i>			
1	0.1	0.06	1.44
5	0.5	0.18	0.86
50	5.0	0.98	0.47
<i>Skin of rats</i>			
1	0.1	0.23	5.52
5	0.5	1.01	4.85
50	5.0	9.55	4.58

observed at inhalation of the aerosol during spraying of plants. Irritation of the mucous membranes of the eyes, nose, and upper respiratory tract is possible; acute poisoning can lead to serious lung disorders. However, due to its low volatility (vapor pressure <10-8 kPa at 25 °C), its flow into the working zone in dangerous concentrations is restricted [2]. Oral poisoning is more common than inhalation. The symptoms of intoxication are nausea, vomiting, chest pain, development of cardiogenic shock, toxic effects on the liver and kidneys. The lethal dose of diquat dibromide for humans is 6-12 g. Deaths after swallowing 20 ml and 50 ml of Reglon (diquat dibromide, 200 g / l) have been reported [8].

Diquat and its preparations have a mild irritant effect on the skin and mucous membranes of the eyes. Prolonged single or multiple exposures with concentrated solutions of diquat led to severe skin and nail damage in personnel [9]. The high prooxidant activity of diquat, which leads to a cytotoxic effect, makes it potentially dangerous when penetrating through the skin.

THE AIM

The toxicological-hygienic assessment of dermal absorption of diquat in terms of potential risk of its bioavailability in professional use.

MATERIALS AND METHODS

The object of the study was cutaneous exposure of diquat, determined in toxicological experiments of different duration (data of scientific literature) and at the stage of state

testing of pesticide preparations based on diquat dibromide (data of a full-scale hygiene experiment, prognostic model of risk assessment).

The methods of expert-analytical study of scientific information on the toxicological properties of diquat, its bioavailability at transdermal admission to the body; and the prognostic model of determination of dermal absorbed dose of diquat for the assessment of the risk of adverse effects on employees are used in the work.

The technical concentrate of diquat dibromide (active substance content not less than 377 g / kg) contains relevant supplements, the content of which is regulated by the Food and Agriculture Organization (FAO), namely: 2,2'-bipyridyl (exhibits mutagenic and teratogenic activity) – not more than 0.75 g / kg, ethylenedibromide (genotoxic action, carcinogenic activity) – not more than 0.01 g / kg, total terpyridine (high toxicity) – not more than 0.001 g / kg.

RESULTS AND DISCUSSION

The toxicological profile of diquat and diquat dibromide is widely reported in the scientific literature [10,11]. The parameters of acute toxicity to rats with different routes of exposure are shown in table I.

As can be seen from the data table, the substance of toxicity is diquat ion. Clinical pattern of laboratory animals acute poisoning is characterized by decrease in motor activity, impaired coordination of movements, with introduction of lethal doses – hypersalivation, hemorrhagic discharge from the eyes and nose, difficult breathing, death of animals occurs on the 3rd -7th days associated with pulmonary

insufficiency. The toxic effect is manifested in changes in the redox enzymes activity, an increase in the number of erythrocytes, leukocytes and reticulocytes, hemoglobin content and sulfhydryl groups of whole blood [10].

In the mode of short-term and long-term exposures, hepatotoxic, nephrotoxic and cataractogenic effects are the main ones in the toxic action of diquat.

The cumulative properties of the diquat are not expressed. The plasma half-life period is 4 hours; more than 90% of the absorbed dose is excreted in urine and bile during 96 hours. The highest content of diquat is in the kidneys, gastrointestinal tract, lungs, and liver, in small amounts it is found in the lens [2].

The absorption of diquat at low concentrations penetration is negligible and it is: in the gastrointestinal tract – 4%, through the skin – 1-2%. The effect of high concentrations, which can exert local irritant effect, increases the degree of absorption. Absorption of diquat from the lungs is more effective (about 70% of the respiratory fraction of the aerosol particles), which results in its high inhalation toxicity.

The literature provides data on the study of penetration of diquat through the skin in vitro using diffusion chambers and isolated skin of rats and humans; and in vivo – studies in rats and volunteers [2,10,12-14].

In studies conducted by Scott R.C. and Corrigan M.A. (1990) using diffusion chambers, diquat was applied to human and rat skin in a volume of 0.1 ml / cm² at various concentrations: 1 mg / ml, 5 mg / ml and 50 mg / ml (skin scraps were taken after death) from the abdomen and the dorsal part of the rat body). A measured volume of 0.9% saline was placed into the receptor chamber. Samples of 50 µl were taken from the receptor chamber at different time intervals and the content of the diquat was also estimated. The uptake period (delay time) was about two hours for rat skin and 15 hours for human skin [12, 13]. A steady state absorption rate was calculated for each dilution as shown in Table II.

As can be seen from the data of table 2, penetration of diquat through human skin is slower, and the percentage of absorbed dose increases towards less concentrated solutions. The total absorption of diquat through human skin at 24 hours was (0.47-1.44)%, through the skin of rats – (4.58-5.52)% of the exposure dose.

According to other data [2,10] dermal absorption of diquat through human skin (in vitro data) from concentrated aqueous solutions of preparative forms (diquat content of dibromide – 200 g / l) was (0.2-0.5)%, from diluted solutions (1 + 100 dilution; 1 + 200 and 1 + 250) – 0.5%, 2% and 8%, respectively.

Dermal absorption of diquat in vivo was investigated in rats with single application of 0.05% aqueous solutions of skin; 0.5; and 5.0 mg of ¹⁴C-diquat (skin exposure – 24 hours). After 24 hours, systemic absorption of diquat was found to be 2.5%, 2.1%, and 3.4% of the applied dose, respectively [10].

In studies conducted by Feldmann R.J. and Maibach H.I. (1974) on human volunteers, single diquat was applied to the forearm area in the amount of 4 mg / cm² [14]. Urine

specimens were collected for 5 days (at 4 and 12 hour intervals on the first day and every 24 hours in the following days) and analyzed for the content of diquat. The total excretion of diquat with urine was 0.3 ± 0.1% of the applied dose, which indicates the bioavailability of diquat when it gets on the skin. When assessing the parameters that affect absorption, it was noted that occlusion increases absorption 3.5 times (up to 1.4%), and damaged skin – 9.5 times (up to 3.8%).

Diquat penetrates cell membranes, mainly due to diffusion, which is known to be facilitated with the ability of the substance to dissolve in lipid and aqueous phases. Diquat, as a highly charged cation, is practically insoluble in the lipid phase (partition coefficient in the n-octanol / water system: log K_{o/w} = – 3.45 at 22 ° C) [2], and therefore it penetrates slowly through membranes. Binding to the anionic sites of different substrates also limits its systemic bioavailability. However, the ability of diquat to activate free radical lipid peroxidation (LPO) causes damage to biological membranes and cell death, which form a barrier to the entry of toxicants into the body (alveoli, mucous membrane of the gastrointestinal tract).

The bioavailability and systemic toxicity of diquat at its long cutaneous exposures is confirmed by the results of toxicological studies obtained in the subacute studies of rats and rabbits [13].

When the authors applied to the skin of Sprague-Dawley rats (12 animals in each group) diquat dibromide (20.64% diquat-ion) at doses of 5, 20, 40, 80 mg-ion / kg bw for 21 days, systemic toxic effects included mortality in groups of animals treated with 20, 40, and 80 mg-ion / kg bw (1/12, 5/12, and 11/12) starting at 18, 8, and 6 days, respectively. Changes in hematological and biochemical parameters associated with dehydration and malnutrition, impaired renal, hepatic and cardiac function have been found in the maximum dose surviving animal. Local manifestations of diarrhea occurred in all experimental groups and included skin irritation (erythema, edema, atony, desquamation) and tissue destruction (necrosis, scab). The frequency and severity of these effects were directly proportional to the dose applied. NOEL for systemic effects made up 5 mg-ion / kg bw, NOEL for local effects hasn't been not established.

In experiments with rabbits (10 animals in each group), when we applied to the skin diquat dibromide at doses of 3.1; 6.3; 12.5 and 25 mg / kg bw, animal mortality was observed in all experimental groups within 20 days: at the highest dose, mortality was 100% (first death was recorded on day 4), at a dose of 12.5 mg / kg – 90% (first death on day 3), dose of 6.3 mg / kg – 20% (first death at day 7), dose of 3.1 mg / kg – 10% (death reported at day 10). The manifestations of systemic effects, the intensity of which depended on the dose applied to the skin, were ulcers of the gastric mucosa, degenerative changes in the convoluted tubules of the kidneys, bleeding in the thymus, plethora of the lungs. The locally marked redness and swelling of the skin were noted as well as with the highest dose introduced – large areas of epidermis necrosis. No invalid level was found during the exploratory study of diquat.

Table III. Estimation of dermal exposure by diquat

Indicator		Value		
<i>Production cycle: mixing / filling (preparation of working solutions)</i>				
Container size / neck tube diameter, l / mm		10 / 45		
The contamination level of the skin (of the hands) during work, ml		0.1		
The consumption rate of the drug, l / ha		2.5		
The maximum cultivated area, ha		50 ra		
Number of work operations per shift		13		
The contamination level of the skin (of the hands) per shift, ml / day		1.3		
Use of personal protective equipment		gloves		
Transfer to skin,%		5		
Dermal exposure to the drug, ml / day		0.065		
<i>Production cycle: Desiccation with a barrel sprayer with hydraulic nozzles</i>				
The flow rate of working fluid, l / ha		300		
The level of contamination by the working solution, ml / h		10		
Distribution of pollution by localization (%)	D _{hand} (65)	D _{body} (10)	D _{leg} (25)	
Use of personal protective equipment	gloves	uniform	working shoes	
Transfer to skin,%	10	5	15	
Dermal exposure to working solution, ml / h	0.65	0.05	0.375	
Total dermal exposure to working solution, ml / day		6.45		

Table IV. Assessment of skin-absorbed dose of diquat

Indicator	Production cycle	
	Mixing / filling	Desiccation
Dermal exposure to the drug and working solution, ml / day	0.065	6.45
The concentration of diquat in the preparation and working solution, mg / ml	200	1.667
Dermal exposure to diquat, mg / day	13	10.75
Dermal absorption of diquat, %	0.5	8
Skin absorbed dose, mg / day	0.065	0.86
Total absorbed dose, mg / kg bw / day	0.0154	

Thus, the results of the study of subacute dermal toxicity of diquat confirm its ability to exhibit a cytotoxic effect, thereby increasing the bioavailability and skin-resorptive toxicity.

Simple calculations using the oral absorption factor of 0.04 [2] for diquat give reason to claim that the dose of the toxicant that entered the bloodstream and caused the death of 50% of laboratory animals is 8.56 mg / kg (4% of a single dose of 214 mg / kg administered to rats). When applied on the skin of rats in the subcutaneous experiment mode, a dose of 40 mg / kg, starting from the 8th day of the experiment, resulted in the death of almost half of the experimental animals (42%) that is the total dose of diquat absorbed through the skin under these experimental conditions was 7 mg / kg.

To sum up, it is especially important to emphasize the repeated impact. Despite the moderate risk of single skin application, repeated and prolonged, low-dose cutaneous exposures occurring under real pesticide conditions can

impair skin barrier function and increase the risk of skin resorption.

The confirmation of this conclusion was expressed in the working studies under the real agricultural modeled situations [15]. To predict and quantify the occupational risk of dermal exposure to diquat, which the operator may experience during mixing / filling (preparation of working solutions) and plant spraying phases, we determined cutaneous exposure doses and dermal absorption values using the main predictive exposure provisions [16].

The calculation was performed for a typical formulation on the basis of diquat dibromide: soluble concentrate, the content of diquat dibromide in the formulation – 200 g / l, the consumption rate of the drug – 2.5 l / ha, the size of the container – 10 l, the diameter of the neck of the container – 45 mm.

According to [2], the AOEL of diquat is 0.0002 mg / kg, dermal absorption from concentrated formulation solutions – 0.5%, from dilute aqueous solutions – 8% (dilution 1 + 250).

Estimated values of dermal exposure to diquat at different stages of production of the pesticide are shown in table III.

The calculations showed that if personal protective equipment is used, the predicted cutaneous exposure dose of diquat for a shift (6 hours) will be: 0.065 ml at the mixing / filling stage (preparation of working solutions), 6.45 at the spraying (desiccation) stage of the plants, ml.

The data of the absorbed dose of diquat are shown in table IV.

As can be seen from Table 4 data, the total dose of diquat absorbed through the skin, including all production cycles, is 0.0154 mg / kg bw / day, exceeding the allowable level (AOEL) by 77 times.

The results suggest that the risk of adverse effects of diquat exerted on personnel is considered to be extremely high, even without an inhalation component, which requires appropriate management decisions to be taken to ensure high level of the human health and environment protection.

In this regard, it should be noted that such a decision has already been taken in the European Union. Thus, in accordance with the EU Regulation No 2018/1532 of 12 October 2018, the marketing authorization for an active substance of diquat has not been renewed and amendments are made to Regulation (EU) No 541/2011 on the conditions for the approval of diquat and the forbidding use of plant protective products (PPPs) with it [17].

The rationale for this decision is confirmed high risk of diquat for operators, observers and residents, which was calculated using validated risk assessment models for all pesticide use conditions. In addition, a high risk of diquat for birds was identified.

In accordance with the Art. 46 of Regulation (EU) No 1107/2009 of the European Parliament and of the Council, the Member States have time period until 4 February 2020 to cancel authorizations for plant protection products containing diquat.

In Ukraine, the decision on the registration of PPPs is made by the relevant state bodies, in compliance with the basic principles of protection against toxic effects of pesticides. The EU-Ukraine Association Agreement requires adoption of common rules and methods for risk assessment in order to preserve human health and protect the environment.

CONCLUSIONS

1. Diquat is a non-selective contact herbicide and desiccant, it belongs to the class of chemical compounds derived from bipyridylum with high pro-oxidant activity and cytotoxic effect.
2. According to the results of empirical and theoretical research of scientific information, the value of dermal absorption of diquat was estimated in various toxicological experiments. Repeated and prolonged, low-dose cutaneous exposures occurring in the actual pesticide application can impair skin barrier function, increase bioavailability and potential occupational risk of skin and resorptive action of diquat.

3. Due to the high risk of adverse effects of diquat on the personnel, general public and environment in the European Union, management decisions have been introduced to forbid the use of plant protection products based on it. The fulfillment of the conditions of the Association Agreement between Ukraine and the European Union indicates the need to develop common regulations and risk assessment methods aimed at ensuring high level of the human health and environment protection.

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ORIGINAL ARTICLE

CLINICAL AND LABORATORY PECULIARITIES OF CHILDREN'S STATE WITH CHRONIC TONSILITIS ON TYPE 1 DIABETES

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ABSTRACT

The aim: Improvement of early diagnostics quality and efficiency of chronic pharyngeal diseases (CPD) treatment in children with DM-1 by studying the clinical and laboratory features of the disease course and complex therapy administration.

Materials and methods: A comprehensive examination of 707 children with diabetes mellitus-1, aged 3 to 18, was conducted, which determined clinical and laboratory features of their state in the presence and absence of chronic tonsillitis (CT).

Results: Using microbiological and cytological examination of the palatine tonsils (PT) crypts, the leading role of microbial associations in the pathogenesis of HT in the formation of immune inflammation was determined, their influence on the local immunity indices was defined. Significant disturbances of the microcirculatory bed (MCB) in the presence of HT in children with DM-1, direct correlation between CPD and the presence of vascular glomeruli, changes in the retinal capillary structure ($r = 0,332$; $p < 0,05$) according to biomicroscopy bulbar conjunctiva (BMBC) data were determined. The study of psycho-emotional state in children with CT and DM-1 was conducted, along with applied psycho-corrective program, which significantly reduces the manifestations of aggressiveness, aggression, and frustration reactions in them.

Conclusions: A non-invasive complex of the study of PT state on changes of local immunity indices, microbiocenosis and cytological data for correction of CPD course was developed, which allows to obtain faster compensation of CT and contributes to patients' quality of life improvement.

KEYWORDS: chronic tonsillitis, children, palatine tonsils, type 1 diabetes

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INTRODUCTION

The incidence of CT, which is especially prevalent among children aged 6 to 17, attracts the increased attention of otolaryngologists with frequent recurrences, development of complications and its influence on diseases of other organs and systems, despite the wide arsenal of modern methods of treatment [1, 2, 3].

Of particular importance is the CT of comorbid course with such autoimmune endocrine disease as DM-1. Disorders of the functional state of PT at CT in children with diabetes mellitus-1 are closely related to the level of glycemic control (GC) and are manifested by increased susceptibility to infections, increased cell adhesion of microorganisms, the presence of micro- and macroangiopathy, neuropathy [4,5].

It also affects the antibodies synthesis, the phagocytic activity of macrophages and leukocytes, the barrier function of the mucous membranes and the regenerative capacity of all tissues, which generally inhibits local defense mechanisms with a decrease in the overall immunoreactivity of the organism [6, 7].

The problem of diseases of ENT-organs in children with diabetes mellitus type 1 (DM-1), has not been studied and is currently relevant and needs urgent solution. According to the literary sources, there are only a few scientific papers

on some diseases of ENT-organs in adults with diabetes mellitus-1. Therefore, we have conducted scientific research for the time in Ukraine, which is devoted to the study of CPD problem in children with DM-1.

THE AIM

The aim of the work is to improve early diagnostics quality and efficiency of chronic pharyngeal diseases (CPD) treatment in children with DM-1 by studying the clinical and laboratory features of the disease course and complex therapy administration.

MATERIALS AND METHODS

During 2013-2018, 707 patients with DM-1 who were hospitalized at the endocrinological wards of the National Children's Specialized Hospital «Ohmatdet» of the Ministry of Health of Ukraine and the City Endocrinological Department of the Children's Clinical Hospital No. 6 of Kyiv were examined. The age of the patients ranged from 3 to 18, 365 boys (51.6%), 342 girls (48.4%), the average age was (10.9 ± 0.4) years.

The main study group consisted of 238 children with CT on the background of DM-1, and comparative groups included children with DM-1 without CT ($n = 60$) and pa-

tients with CT without DM-1 ($n = 20$). The control group included 20 practically healthy individuals. The distribution of patients by gender and age in all compared groups was similar in terms of indexed and initial clinical characteristics.

Microbiological and cytological research was conducted in accordance with the current regulatory documents by accredited methods; quantitative assessment of microbiocenosis was defined on the basis of penetrance indices, population level of bacteria when calculating colony forming units in 1 g of material (lg CFU / g).

Immunological research methods were carried out in accordance with current guidelines and instructions of the manufacturer of the reagents. Calculation of the concentration of immunoglobulins was carried out by the enzyme immunoassay on the Stat Fax 2100 analyzer (USA): the content of secretory immunoglobulin A (sIgA), the level of α -interferon (α -INF) by the enzyme immunoassay (Vector Best, Russia) were studied; concentration of interleukin 1 β (L-1 β), lactoferrin was studied by enzyme-linked immunosorbent assay (Protein Contour LLC, Russia).

The study of the MCB status in children with DM-1, and its influence on the development and course of CPD was carried out by defining the nail bed capillaroscopy (NBC) indices according to the standard method, biomicroscopy of the bulbar conjunctiva (BMBC), which was assessed by using slit cleft followed by photoregistration of vessels and microrinoscopy (MCRS). The results of the BMBC examination included the assessment of the caliber of microvessels, their course, myandric tortuosity of capillaries, venules, arterioles, arterial-venous ratio, number of functioning capillaries, size of vascular patches, rate and transparency of blood vessels, degree of transparency of blood vessels, degree of transparency, perivascular edema, hemorrhage, sweet phenomenon.

The credibility of differences between the comparison groups was determined by the Student's parametric criterion and the non-parametric Mann-Whitney test. The difference between the indices was considered reliable at $p < 0.05$. Fisher's angular transformation method was applied to detect differences between the two groups with minimum sample values and to compare with the qualitative indices expressed as a percentage.

Correlation and multivariate analysis of the researched indices was carried out with the calculation of the Pearson correlation coefficients (r). Statistical calculations were performed by IBM SPSS 17.0 software, Statistic 8, Microsoft Office Excel 2010.

The experts session of the Ethics Committee of the Shupyk National Medical Academy of Postgraduate Education approved this clinical study and gave permission to conduct it in accordance with the current legislation of Ukraine, modern ethical standards and principles of conducting clinical research (Minutes of the session of the Ethics Committee №10 dated 06/10/2014).

RESULTS

According to the results of otorhinolaryngological examination of children with diabetes mellitus-1, the structure

of diseases of ENT organs specific for them was obtained. The largest group among them was patients diagnosed with CPD ($n = 424$): the largest group of children with CT (238; 56.2%), consisting of 136 boys, 102 girls aged 6-18. Hypertrophy of adenoid vegetations was diagnosed in 21.2% ($n = 90$) of the examined, hypertrophy of the tonsils – 8% ($n = 34$), chronic adenoiditis – 7.5% ($n = 32$), chronic pharyngitis – 7.1% ($n = 30$).

We have found a non-angina form of CT, which is more commonly diagnosed in older school-age children (34.8%) than younger children (26.9%). Roller-like thickening of the edges of the anterior and posterior palatal brackets was established in 77.6% of younger school-age children versus 74.4% – older, a congestive hyperemia stripe in the front brace – 64.4% versus 34.8%, the presence of scar adhesions between the tonsils and palatine brackets – 73.6% versus 43.0%, the presence of caseous plugs in the lacunae of TC during rotation in children aged 6-12 is significantly ($p < 0.05$) higher (71.0%) compared with patients aged 13-18 (44, 1%), however, the presence of liquid pus in PT lacunas during rotation in them is significantly ($p < 0.05$) lower (7.9%) compared with patients aged 13-18 (23.2%), augmentation and consolidation of regional lymph nodes in children of both groups were not significantly different (47.3% versus 56.9%).

Diagnosis of DM-1 was established by doctors of the endocrinological department, who were assessing GC state in the examined children by determining glycated hemoglobin (HbA_{1c} (%)) and indices of glycemia. The ground for grouping by GC level was based on the value of HbA_{1c} (%) in accordance with the Protocol of providing medical care to children with diabetes (Order of the Ministry of Health of Ukraine No. 254 of 27.04.2006). Optimal (GCO) was considered in the case of the level of HbA_{1c} in the range of 6-7.9%, suboptimal (GCSO) – 7.9-9%, high-risk GC (GCHR) – more than 9%. Glucose levels in the examined patients ranged from 3.8 mmol / l to 17.2 mmol / l (average 10.5 mmol / l). In 63.2% of the examined children, GCHR was observed, 24.4% of the examined children had GCSO, and only 12.4% of them had GCO.

Microbiological studies of PT lacunae revealed that the studied microbiocenoses in children with DM-1 mainly consisted of mono- and multicomponent associations of aerobic and facultative anaerobic microflora. 2-3-component associations were found to be 2.5 times more frequent in CT in patients with DM-1 than in individuals with DM-1 without CT. According to the research done, no significant differences were observed between the persistence of microbiocenoses consisting of 2–3 microorganisms in the main and comparative groups, however, microbiocenoses that included 2 or more representatives were detected in 50% of patients in both groups ($p < 0.05$).

Representatives of the enterococci *Klebsiella*, *Enterobacter*, *E. coli* persisted in the children of the main group much more often than in comparative group patients, in an amount exceeding lg 3 CFU / g. This was especially true of children in the main group, where the *Klebsiella pneumoniae* strain was sown 3.5 times more often than in

the comparative one. Given pathogen was sown in 22% of cases in the amount of $10^{4.01-5.41}$ CFU / g. Yeast mushroom colonization in both groups was represented equally in the amount of $10^{3.5}$ CFU / g.

According to the results of the cytological study from the PT lacunae in children of the main group, the following cytomorphological features were identified.

1. The low level of cellular composition indices – characterized by a moderate or high degree of desquamation of the flat epithelium with the signs of cell proliferation and degenerative changes. Giant multinucleated cells of chronic inflammation are detected. Neutrophilic leukocytes with incomplete phagocytosis of bacteria are in large numbers. Macrophage locations are found. Histiocytes are in small numbers. Lymphocytes are rare or absent. The bacterial flora is mixed, in particular spirochetes, fusobacteria are in large numbers, fungus are present.
2. The average level of cellular composition indices – the preparation is dominated by active lymphocytes, part in a state of ballast transformation. Plasmacytes are present, separately and in clusters. Neutrophilic leukocytes are in small numbers. Cells of the flat epithelium, individually and in layers are in small numbers. Mixed bacterial flora is in a small amount.
3. The high level of cellular composition indices- characterized by a large number of active lymphocytes in the preparation content, separately and in clusters, partly in a state of ballast transformation, places of mitotically divided cells. Neutrophilic leukocytes are in small numbers. Plasmacytes are present, separately and in clusters. Moderate desquamation of the flat epithelium is observed. Mixed bacterial flora is in a moderate amount.

Cytological data analysis in children of the main group, considering GC level, has found a statistically significant difference ($p < 0.01$) in reduction of high level in cell composition (5.3%) compared with the average (45.6%) and low (49.1 %) levels at GCHR. A similar tendency was observed at GCSO, where a high level of cellular composition (12%) was significantly ($p < 0.05$) reduced compared with average (36%) and low (52%) levels. At CT in children with DM-1 and GKO, the indice of high cellular composition is significantly lower (7.6%) compared with the average (46.3%) and low (46.1%) levels ($p < 0.05$).

Regarding the course of CT with frequent and rare exacerbations in children with DM-1, the following features of the cytological state have been identified. In patients with CT and frequent exacerbations on the background of DM-1, the high level indice of cellular composition was reduced (5.1%) and had a statistically significant difference ($p < 0.01$) compared with the moderate (45.7%) and low (48, 2%) levels. At CT with rare exacerbations (up to 3 times), the high level of cellular composition was also significantly ($p < 0.05$) lower (12.4%) compared with the moderate (40.1%) and low (47.5%) levels.

We have conducted immunological research of local immunity and found that humoral mechanisms of oropharyngeal secretion (OPS) protection at CT in children with

DM-1 also had specific features. Thus, the concentration of sIgA in OPS of children with DM-1 was higher than normal (57-260 μg / ml), the lactoferrin content in the main group was lower (9224 ng / ml) compared with the children of the control group (10858 ng / ml), and the concentration of α -INF in OPS in patients of the main group was 12.0 pg / ml, which was significantly higher- more than twice than in the comparative group (7.8 pg / ml) ($p < 0.05$), however, the level γ -INF in children of the main (332 pg / ml) and comparative (322 pg / ml) groups had no significant differences. The level of proinflammatory -L-1 β in OPS of the children of the main group (49.9 pg / ml) was twice lower than in the comparative group (108.4 pg / ml) ($p < 0.05$), and the concentration of lysozyme in the OPS in patients with CT and with DM-1 was 3.0 μg / ml compared with 2.1 μg / ml in the comparative group.

In the study of MCB state indices in the group of patients examined, it was found that less than 1/3 of children with CT on the background of DM-1 have a normal capillary structure in contrast to half of the patients in the comparative group ($p < 0.05$). In addition, there was a clear tendency of the increase of the patients number with severe microcirculatory disorders in the main group. II-III degrees of MCB disorders in children of the main group were detected in 22.5-27.5% of cases, while in comparative group II degree was 14.5%, III – only 7.1% ($p < 0.05$), which is 3 times less than with the CT presence.

In assessing the results of BMBC in patients with DM-1, the dependence of vascular disorders from the nature of ENT pathology was detected, especially at CPD. Thus, in the presence of CT in children on the background of CD-1 vascular disorders in the form of irregularity of the caliber of vessels (45%), single microaneurysms (65%), myandric tortuosity of capillaries (80%) and venules (50%), single arterio-venular anastomoses (35%) were revealed. Extravascular abnormalities in the form of microhemorrhages (15%) and perivascular edema (10%) were also observed.

We found that with longer duration of disease (3.68 ± 0.05); $p < 0.05$) in children with CD-1, in 75.3% of cases chronic pathology of ENT-organs prevails, among which CT is the leading place (48.5%). While studying the assessment of the severity of DM-1 by the level of GC, the influence of CPD on the increase of HbA1s was found in the examined children, with 61.4% of such patients having a GCHR level.

According to the data of conducted correlation analysis of changes in MCB indices, a direct connection between the presence of chronic ENT pathology and vascular glomeruli ($r = 0.529$; $p < 0.05$), changes in the retinal capillary structure ($r = 0.332$; $p < 0.05$) according to BMBC data, GC levels and the presence of abnormal mucous vessels of the NS mucosa ($r = 0.488$; $p < 0.05$) was detected in patients with DM-1, which confirms the negative influence of the underlying disease on disorders of MCB, especially in the presence with CT.

According to BMBC data, a direct correlation between MCRS indices was detected, such as mucosal dryness, and the presence of multiple microangiopathies ($r = 0.349$;

$p < 0.05$), venous sacculation ($r = 0.320$; $p < 0.05$), between the abnormal vessels and the presence of vascular glomeruli ($r = 0.459$; $p < 0.05$), irregularity of the vessels caliber ($r = 0.329$; $p < 0.05$), venous sacculation ($r = 0.375$; $p < 0.05$). In addition, the indice of capillary misalignment was directly correlated with the presence of vascular glomeruli ($r = 0.378$; $p < 0.05$).

Extravascular abnormalities in the form of hemorrhages, which was detected at MCRC in the children of the main group were significantly correlated with the meandric tortuosity of the capillaries ($r = 0.314$; $p < 0.05$) and the presence of abnormal vessels ($r = 0.378$; $p < 0.05$).

Research data of the psycho-emotional state of patients with CT on the background of DM-1 found the presence of aggression mainly against adults (76.2%), low self-esteem (66.7%), fatigue, exhaustion (64.3%), and 56, 8% of children experienced fears, feelings of insecurity and anxiety. Only 17.3% of patients had a positive emotional state, characterized by emotional satisfaction, desire to communicate.

Having analyzed the results obtained in the examined children with CT and DM-1, we concluded that it is necessary to apply a psycho-corrective program for this category of individuals. After conducting the corrective program in patients according to our method, the average aggressiveness in the children of the main group decreased significantly ($p < 0.05$) from 81,6% to 26,6%, which was not observed in the comparative group; manifestations of verbal (from 37.8% to 17.7%; $p < 0.05$) and spontaneous (from 24.4% to 13.3%; $p < 0.05$) aggression significantly decreased the number of times. Also in the main group aggressive self-defense (from 37.8% to 11.1%; $p < 0.05$) and indicators of frustration reactions (from 64.4% to 24.4%; $p < 0.01$) decreased, but no significant changes were observed in the children of the comparative group.

DISCUSSION

The fact that the value of GC level (HbA1c) at CT in the patients of the main group is statistically significantly bigger than the corresponding value in the comparator may indicate a significant worsening of the DM-1 course in children with CT.

The insemination of PT with gram-positive coccal flora with high biological activity in such patients is indisputable, however, conditionally pathogenic bacteria value with pronounced persistent potential, which is combined with high indices of colonization intensity, increases in them.

The microbiocenotic peculiarities of PT in children of the main group which were investigated and revealed by us indicate the necessity and expediency of inclusion of methods and antiseptic agents in the regimens of treatment that have a directed antimicrobial effect, and can also ensure the restoration and preservation of normal microbiocenosis of PT.

It should be noted that cytological study of the content of PT lacunas at CT in children with DM-1 conducted by us is an additional informative method for assessing the state of PT. The content of the crypt is a source of constant antigenic and mitogenic stimulation necessary to support the body's

immunological homeostasis. Research of the cellular composition of smears from lacunae, especially in cases with no pronounced clinical symptoms, allows the otolaryngologist to define the form of CT more accurately, to assess the effectiveness of the performed conservative treatment.

However, conducting such a standard bacteriological and cytological research in children with DM-1 has certain complicated features, given the physiological and psychological characteristics of such patients. At the same time, the high percentage of identification of characteristic changes in PT state, the speed and convenience of its conducting make it possible to consider this method as promising for the screening of PT state disorders for an earlier beginning of treatment and rehabilitation measures. [8]

Changes in local immunity revealed by us in the research of OPS at CT in children on the background of CD-1 showed a pronounced immunological deficiency that requires correction. The role of immune mechanisms, in particular chronic inflammation, in the development of DM-1 and its vascular complications has been actively discussed in recent years. The presence of chronic inflammation in PT in these patients suggests that this process is directly related to the violation of the immune status and the formation of early complications. Immune system cells (lymphocytes, monocytes) and endothelium are involved in the development of the inflammatory process, while at the same time intercellular interactions are performed by adhesion molecules, cytokines, and growth factors. [9,10] Therefore, immunoinflammatory reactions are involved in the development of autoimmune destruction of pancreatic islets at DM-1, as well as in the formation of microangiopathy.

Therefore, the study of MCB status in the comorbid course of CT in children with DM-1 is an important step in the laboratory research, which complements the findings of previous researchers. [11]

Thus, the data of our correlation analysis proved that at CT in children on the background of DM-1, infectious-inflammatory changes of the mucous membrane of the NS lead to disorders of the peripheral trophic of its tissues and worsen the MCB, which requires early diagnosis and administration of effective therapy.

The data of examination of the psycho-emotional state of patients with CT on the basis of the psycho-diagnostic methods used by us, considering the severity and duration of DM-1 disease, confirmed the opinion of specialists that significant changes in the emotional sphere are taking place in such patients. [12]

Therefore, this contingent of patients, especially those ones belonging to the group with low level of adaptation, requires special psychological support.

CONCLUSIONS

At CT in children with CD-1, the density of microbial insemination of PT lacuna is $lg(3.86 \pm 0.78)$ CFU / g compared to patients with DM-1 without CT ($lg(3.35 \pm 0.43)$ CFU / g; $p = 0.05$). Microbiocenoses of TC, which include 2 or more representatives of conditionally pathogenic microorganisms,

are credibly detected in 50% of the children of the main group compared with the comparative ($p < 0.05$), and their species composition is expanded due to the persistence of *Staphylococcus aureus* (61%), *Klebsiella pneumoniae* (22%), *Streptococcus pyogenes* (12%).

Specific cytologic features of the content of PT lacunas at CT in children on the background of DM-1 with GCHR are a significant ($p < 0.01$) increase of low (49.1%) and medium (45.6%) levels of cellular composition compared with high (5, 3%) cell level.

Humoral mechanisms of OPS protection in children with CT and DM-1 are distinguished by a decrease in the level of sIgA, lactoferrin, and a twofold decrease in the level of α -INF compared with the comparative group, indicating an immunological imbalance that requires correction.

Based on the correlation analysis of microcirculation indices, a direct correlation between the presence of chronic ENT pathology and changes in the retinal capillary structure ($r = 0.332$; $p < 0.05$) was detected according to BMBC data. A direct correlation between the level of GC and the presence of abnormal vessels of the nasal septal mucosa ($r = 0.488$; $p < 0.05$) at HT in children with DM-1 confirms the negative influence of the underlying disease on the disorder of the MCB state.

Psycho-emotional disorders in children with CT on the background of DM-1 are characterized by the presence of fears, feelings of insecurity and anxiety in 56.8% of patients, fatigue and exhaustion - 64.3%, low self-esteem - 66.7%. The identified changes require the application of a psycho-correction program that can successfully improve the patients' psychological state.

Considering the above results of our research, the study of CPD in children on the background of DM-1 is expedient and has important social and practical value in clinical medicine. That is the multidisciplinary approach and the cooperation of specialists of different profiles that will significantly improve the quality of life of children suffering from CT and DM-1.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

EFFICACY OF PECTORAL NERVE BLOCK TYPE II VERSUS THORACIC PARAVERTEBRAL BLOCK FOR ANALGESIA IN BREAST CANCER SURGERY

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INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy among women in the regions with different economic development with an estimated 1.67 million new cancer cases diagnosed in 2012 [1]. In Ukraine these cancers are also the first most common malignancies among women. And in last decades its incidence has increased [2]. The main surgical method of treatment for operable breast cancer is organ-sparing surgery with lymph node dissection. Despite the variety of methods of analgesia, up to 40% of women suffer from acute pain after this surgery [3]. There is a high risk of chronic pain development after this surgery [4]. Therefore, research and development of safe analgesia techniques for these interventions remains a topic of interest.

Widespread use of the ultrasound visualization for the peripheral nerve blockade has given an impetus to the development of new, minimally invasive myofascial blocks. Particularly, in the last few years for the chest wall anesthesia has been proposed the erector spinae plane block, serratus anterior plane block, pectoral nerve block types I and II and transversus thoracic muscle plane block. All of them currently are considered as simple to perform, superficial and safe blocks, and have a small number of contraindications. However, during several decades for analgesia in

the breast surgery more invasive techniques, in particular, thoracic epidural analgesia, paravertebral and intercostal nerve blocks, have been effectively used. Actually, the thoracic paravertebral block provides good perioperative analgesia [5], and it can be used even as monoanesthesia, when block is performing at multiple vertebral levels [6]. The main disadvantage of this method is the risk of serious complications, such as pneumothorax and administration of local anesthetic in the subarachnoid space. There is no clear answer to the question whether minimally invasive pectoral nerve block type II (Pecs block) can be as effective as thoracic paravertebral block (TPVB) for perioperative analgesia. Several studies show that pectoral nerve block reduces the need for opioid analgesics in the postoperative period compared with paravertebral block [7]. On the other hand, some studies have shown that TPVB has more powerful analgesic effect than Pecs block [8]. Versyck et al. performed the meta-analysis of five studies, which shows that analgesic effect of these two methods is comparable [9].

THE AIM

The aim to compare the efficacy of pectoral nerve block type II and thoracic paravertebral block for analgesia during and after breast cancer surgery.

MATERIALS AND METHODS

The study was conducted from January 2018 to May 2019 in the Feofaniya Clinical Hospital — the clinical site of NMAPE's Department of Anesthesia and Intensive Care. The work plan was approved by the institutional ethics committees. Female patients in the age group of 18-80 years with ASA grade I–II, who were undergoing elective mastectomy or quadrantectomy with axillary lymph node dissection, were included in this study. Also we used exclusion criteria, such as allergy to local anesthetics; a body mass index of more than 35 kg/m²; pregnant women; patients with chronic pain in the arm and/or chest, who constantly use painkillers and/or other medications to treat chronic pain; patients who were previously operated on this mammary gland and/or received radiotherapy; patients who use anticoagulants or have a bleeding disorders; skin inflammation at the local anesthetic injection site.

Patients were randomized (1:1 ratio) into two groups: pectoral nerve block (Pec) and paravertebral block (TPVB). The perioperative procedures in both groups were similar and consistent with the Feofaniya Hospital's local protocols of preparation, examination and. After admission to the operating room, adequate intravenous access and standard monitoring including pulse oximetry, non-invasive blood pressure measurement, ECG, capnography and BIS-monitoring were established for the patient. After establishing a peripheral intravenous line diazepam 5 mg and non-steroidal anti-inflammatory drug (ketoprofen 100 mg) were administered for premedication. Then appropriate block was performed in aseptic settings and under ultrasound guidance.

Pectoral nerve block was performed with the technique described by Blanco et al. [10]. The ultrasound machine "General electric Logiq E" (GE Healthcare, Little Chalfont, United Kingdom) with a linear probe (8-15MH) was used to visualize the anatomical structures. Probe was placed in the subclavian area perpendicular to the middle of the clavicle, where we visualized the pectoral muscles (major and minor), subclavian artery and subclavian vein. Then we moved the probe toward the armpit and at the level of the fourth rib, after achieving the optimal ultrasound image, in the aseptic settings and after skin infiltration anesthesia with lidocaine 1%, we injected ropivacaine 0.375% 20 ml between the pectoralis minor and serratus anterior muscles, using in-plane technique and the needle Stimuplex D 50 mm (B. Braun Melsungen AG, Germany). Then, after pulling up the needle, ropivacaine 0.375% 10 ml was injected into the space between the pectoralis major and minor muscles.

The paravertebral block was performed with the patient in the sitting position, at the level of 3-4 thoracic vertebra under ultrasound guidance. The linear probe of the ultrasound machine General electric Logiq E (GE Healthcare, Little Chalfont, United Kingdom) was placed parallel to the spinous processes, 2.5-3 cm from the midline in the direction of the affected side. Then, under all aseptic precautions and after skin infiltration anesthesia with lidocaine 1%, using in-plane technique and the needle Stimuplex D 50 mm (B. Braun Melsungen AG, Germany), ropivacaine 0.5% 20 ml was slowly injected into the paravertebral space.

During and after performing the block, hemodynamic parameters (blood pressure, heart rate), respiration (capillary blood saturation, respiratory rate) and the patient's subjective sensations were monitored to control the intravascular administration of the local anesthetic or its system resorptive effects. The time for performing the blocks, including all aseptic precautions, was recorded. After the block's procedure, the loss of tactile sensitivity in the dermatomes from T1 to T8 and accordingly to the area of local anesthetic distribution was assessed by pin-pricking with a sterile needle every 5 minutes while the patient was conscious. General anesthesia was induced following 15-20 minutes with injection of fentanyl 1-2 mg/kg and propofol 2 mg/kg. Airway patency was maintained by laryngeal mask. Total intravenous anesthesia was supported by continuous infusion of the propofol. BIS-monitoring values were maintained between 40 and 60. Additional fentanyl 50 µg IV boluses were administered when we observed patient's motor reactions, or when heart rate or systolic blood pressure has increased for more than 20% from the baseline, or when BIS values indicated that patient requires deepening of anesthesia. After awakening patients were transported into the ward. Ketoprofen 100 mg was administered for pain relief when pain intensity was greater than 3 points by the Numerical rating scale (NRS) or after the patient's request. If after the ketoprofen administration the pain intensity remained above 3 points or if patient subjectively was unsatisfying from this analgesia, then opioid analgesic (promedol 2% 20 mg) was administered intramuscular for pain relief.

During the operation, the total doses of fentanyl and propofol were noted. Postoperative pain was evaluated using a Numerical rating scale (NRS, 0-10 points; 0=no pain and 10=worst imaginable pain) at rest at 0, 1, 2, 6, 12, 18 and 24 hours after surgery (if the patient slept at the scheduled time, the assessment was not conducted). Also were noted the needs for ketoprofen and promedol and the time to the first administration of analgesics. Postoperative nausea and vomiting was evaluated using a four-point numerical scale (1=no nausea, 2=mild nausea, 3=single vomiting, 4=multiple vomiting). Also were noted the time of first meal and getting the patient out of bed. All possible block-related complications were recorded too.

The obtained data were entered into a Microsoft Excel 2007 spreadsheet (Microsoft Corporation, USA). Program "STATISTICA 10.0" (StatSoft Inc., USA) was used for statistical analysis. The Shapiro-Wilk test was used to check the normality of quantitative data's distribution. In the study non-normally distributed quantitative data are presented as medians and interquartile intervals. Normally distributed quantitative data in the study are presented as means and squared deviation from the mean. The Mann-Whitney U test was used to determine the significance of the differences between groups (i.e., P-value). The categorical data are presented as numbers and percentages, and the significance of the differences between groups was calculated using the Pearson's chi-square test. P-value < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Sixty patients were enrolled in the study and randomized into two equal groups with no statistically significant differences in age, weight and duration of surgery. Quadrantectomy/mastectomy ratio was 14/16 in the Pecs group and 13/17 in the TPVB group (Table I).

The duration of the performing the pectoral nerve block was 14 (12-16) minutes, paravertebral block — 12 (10-15) minutes ($P>0.05$). Pectoral nerve block covered 5 (4.0-6.0) dermatomes, paravertebral — 3 (2.0-4.0) ($P<0.05$). Propofol doses were comparable in both groups: 5.6 (4.7-7.16) and 5.8 (4.4-7.56) mg/kg/h, respectively ($P>0.05$). The intraoperative need for fentanyl was slightly less in the TPVB group — 1.9 (1.63-2.55) versus 2.2 (1.81-2.81) mcg/kg/h ($P>0.05$).

The difference of pain intensity in the postoperative period, assessed with Numerical Rating Scale, was not statistically significant between two groups (Table II).

The number of patients, who did not require any analgesia after surgery, was 9 in the Pecs group and 10 in TPVB group ($P>0.05$). Although the need for analgesia with ketoprofen and promedol was lower and the time to first analgesic administration was longer in the Pecs group compared with TPVB group, but the differences were not statistically significant: 66.7 (± 66.09) and 95.8 (± 90.78) mg ($P>0.05$), 4.0 (± 8.14) and 5.0 (± 8.85) mg ($P>0.05$), 540 (455.0-600.0) and 515 (265.0-650.0) min ($P>0.05$), respectively. Patients got out of bed and began drinking/eating faster in the Pecs group than in the TPVB group: 150 (120.0-240.0) and 180 (120.0-240.0) min ($P>0.05$), 165 (120.0-240.0) and 180 (120.0-240.0) min ($P>0.05$), respectively. The rates and severity (max score — 3 points) of nausea were higher in the TPVB group: 5 vs 2 patients respectively ($P>0.05$) (Table III).

The block-related complications: in the postoperative period one patient from the TPVB group had signs of local anesthetic system toxicity effects including convulsions and lowering of consciousness, which passed quickly after appropriate treatment; also one patient had a significant blood pressure decreasing and required norepinephrine infusion during surgery and in the early postoperative period. There were no complications in the Pecs group.

The innervation of the thoracic wall and axilla region is quite complex and according to modern concepts is provided by several groups of nerves: 1) anterior and lateral skin branches of the 2-6 intercostal nerves and branches of the first and second intercostal nerves (n. intercostobrachialis) (Th1-6); 2) the nerves from the brachial plexus — the medial (C8-T1) and lateral (C5-C7) pectoral nerves, n. thoracodorsalis (C6-C8) and n. thoracicus longus (C5-C7); 3) supraclavicular branches of the cervical plexus (C3-C4).

In the study we compared the analgesic effects of two thoracic wall nerve blocks, as TPVB is well-researched and effective method and Pecs block may be no less effective, but currently has a smaller evidence base. In this study the paravertebral block was performed at only one Th 3-4 level, as well as in most previous researches, which compared the efficacy of these two techniques [7,11,12,13]. Although

there is evidence that analgesic effect is better when the paravertebral block is performed at several levels [14].

The body's areas that are blocked by Pecs block and TPVB are slightly different. After administration into the paravertebral space at the Th3-Th4 level, local anesthetic spreads more in the caudal and less in the cranial directions [15], and blocks the intercostal nerves, their anterior and lateral skin branches respectively. In this case local anesthetic does not block the brachial plexus and supraclavicular nerves. Kulhari et al. reported that after single-shot administration 0.5% ropivacaine 25 ml caused a sensory block for a median of three segments [12], and Cheema et al. reported that 0.5% bupivacaine 15 ml blocked five segments [16]. In our study the median spread of anesthesia was also three segments for paravertebral block.

After performing the pectoral nerve block and the local anesthetic injection between the pectoralis major and minor muscles and between the pectoralis minor and serratus anterior muscles, we can expect the block of four-six lateral cutaneous branches of the intercostal nerves and four nerves from the brachial plexus (n. thoracodorsalis, n. thoracicus longus, nn. pectoralis lateralis and medialis). An additional important factor is that local anesthetic moves into the axillary fossa, provides an analgesia of the axillary lymph node dissection area and blocks nn. intercostobrachialis (T1-T2). This block does not anesthetize the region of supraclavicular nerves and intercostal's nerves anterior skin branches innervation. Blanco et al. described that pectoral nerve block anesthetized four segments, which sometimes varied up to six segments [10]. Kulhari et al. reported that after the injection of 15 ml of anesthetic the sensor block reached 4 segments [12]. In our study the median spread of anesthesia was 5 segments, which may be due to the local anesthetic administration as much as possible caudally over the fourth rib; also we used larger volume of local anesthetic (20 ml) than Kulhari et al.

The type of the anesthetic spread is related with the blocks' analgesic efficacy. In the paravertebral block group patients did not have an adequate analgesia in the axillary region, thus they required more opioid analgesics during and after major surgeries such as mastectomy. On the other hand, pectoral nerve block provided effective analgesia for axillary lymph node dissection and adequately anesthetized the lateral part of the mammary gland, but it did not work when the surgeons performed incision more medially and not always worked when incision involved the lower dermatomes.

El-Sheikh et al. and Wahba et al. reported about the statistically significant decreasing of the intraoperative need for fentanyl after the Pecs block compared with paravertebral block [11, 7]. At the same time, based on the meta-analysis of seven studies, which was performed by Versyck et al., the intraoperative need for opioids was the similar after Pecs block compared to the total anesthesia without any regional techniques [9]. In our study slightly less need for fentanyl during surgery was observed in the paravertebral block group — 1.9 (1.63-2.55) mcg/kg/h compared to the Pecs block — 2.23 (1.81-2.81) mcg/kg/h, but this difference

Table I. Demographic data and operative data.

	Pecs group	TPVB group	P-value
n	30	30	
Age	60,5 (47,0-67,0)	57,0 (45,0-62,0)	0,1973
Weight	74,0 (64,0-83,0)	74,0 (62,0-86,0)	0,8858
Mastectomy/quadrantectomy	14/16	13/17	0,7952
ASA I/II	11/19	9/21	0,5839
Duration of operation, min	100,0 (85,0-125,0)	120,0 (97,5-140,0)	0,0796

Continuous data are presented as median (interquartile range). Categorical variables are presented as number. ASA = American Society of Anesthesiologists.

Table II. Postoperative pain scores (Numerical rating scale).

	Pecs group	TPVB group	P-value
NRS 0	1 (0-1)	0 (0-1)	0,2249
NRS 1	1 (1-2)	1 (1-1)	0,1874
NRS 2	1,5 (1-3)	1 (1-2)	0,4021
NRS 6	1,5 (1-3)	2 (1-3)	0,6367
NRS 12	2 (1-3)	2 (1-3,5)	0,8615
NRS 18	1,5 (1-3)	2 (1-2)	0,4933
NRS 24	2 (1-2,5)	2 (1-2)	0,9618

Data are expressed as the median (interquartile range)

Table III. Postoperative analgesic consumption, duration of the block procedure, dermatomal spread, rate of nausea.

	Pecs group	TPVB group	P-value
Duration of the block procedure, min	14,0 (12,0-16,0)	12,0 (10,0-15,0)	0,1523
Number of involved dermatomes	5,0 (4,0-6,0)	3,0 (2,0-4,0)	0,0044
Propofol, mg/kg/h	5,62 (4,73-7,16)	5,81 (4,39-7,56)	0,7910
Fentanyl, mcg/kg/h	2,2 (1,81- 2,81)	1,9 (1,63- 2,25)	0,2956
Promedol, mg	4,0 ($\pm$ 8,14)	5,0 ($\pm$ 8,85)	0,6723
Ketoprofen, mg	66,7 ($\pm$ 66,09)	95,8 ($\pm$ 90,78)	0,2792
Without analgesia	9/30 (30%)	10/30 (33%)	0,7932
Time to first analgesia, min	540 (455,0-600,0)	515 (265,0-650,0)	0,6773
Time to patient's activation	150 (120,0-240,0)	180 (120,0-240,0)	0,5865
Time to first meal, min	165 (90,0-240,0)	180 (120,0-240,0)	0,5919
Nausea, score 1/2/3/4 (number of patients)	28/2/0/0	25/3/2/0	0,2291
Chi-square test			

Continuous data are expressed as the median (interquartile range) or as mean ($\pm$ squared deviation). Categorical variables are presented as number (percents).

was not statistically significant. The propofol doses were similar in both groups (Pecs/TPVB): 5.6 (4.7-7.16) and 5.8 (4.4-7.56) mg/kg/h respectively and were close to the sedative doses.

The pain intensity at rest, assessed using a NRS, was comparable in both groups during the first 24 hours after surgery (Table II). Heterogeneous data about pain intensity were obtained in previous studies. Syal et al. obtained results with significantly higher scores of the pain intensity in the pectoral nerve block group, but it should be noted that in this study they injected for block only 20 ml of local anesthetic instead of 30 ml [8]. Kulhari et al. reported the same pain intensity in both groups [12]. Wahba et al. in

their study observed the lower NRS scores during the first 12 hours after surgery in the pectoral nerve block group, but during the period from 12 to 24 hours the results were conversely. In general, we can note that in our study the pain intensity was lower than in similar ones, which may be related to the women's ethnic and cultural characteristics in our region.

Breast cancer surgery is not associated with a severe postoperative pain syndrome. Gerbershagen et al. in their study have compared the pain intensity during the first day after 179 types of surgery intervention. Breast cancer surgeries, such as mastectomy and quadrantectomy with axillary lymph node dissection, were at 146 and 160 places

[17]. In our study 9 women in the Pecs group (30%) and 10 women in the TPVB (33%) did not require any analgesia during the whole postoperative period. But comparing the groups, the need for opioid and non-opioid analgesics in the postoperative period was less in the Pecs group, although without a statistically significant difference. In the most currently available studies the postoperative need for opioids (patient-controlled morphine analgesia) was higher in the TPVB group, with statistical significance in two studies [7,12] and without statistical significance in another one [11]. In only one study of Syal et al., which compared fentanyl analgesia in the postoperative period, there was a greater need of opioids in Pecs group, but without statistical significance [8].

The time to first rescue analgesia was longer in the Pecs group and the same results were also observed in other similar studies [7,12,11,13]. In previous studies the time after surgery to the first rescue analgesia varied due to the different plans of postoperative analgesia according to the study design. In our study we evaluated the time to first administration either opioid or non-opioid analgesics.

The rate and intensity of postoperative nausea was higher in the TPVB group, but without statistical significance. There were two patients with nausea (assessed as 2 points) in the Pecs group, and five patients in the TPVB group (two of them had 3-points nausea). Patients in the Pecs group had earlier started getting out of bed and eating, but these findings also were not statistically significant.

As well as in previous studies [7,12,11,8], no complications were observed after pectoral nerve blocks. Instead, in the TPVB group two patients had block-related complications. One patient experienced a system resorptive effect of local anesthetic, although we did not exceed the recommended safe doses for ropivacaine. The convulsions occurred after patient awakening during transportation from the operating room, and were successfully treated by administration of diazepam 10 mg. Then this patient was under observation until the next day morning and she had not any health-related problems. Another patient had hypotension, which occurred after 15 min since the block performing, at the beginning of the general anesthesia induction and required correction with small doses of norepinephrine during the surgery and during the first two hours after intervention. Tahiri et al. in their meta-analysis reported that hypotension and bradycardia are the most common complications of the paravertebral block in breast surgery, and the overall rate of complications, including pneumothorax and epidural local anesthetic spreading, reaches up to 12% [18]. In our study complications rate was 6.7%. Thus the safety profile of the pectoral nerve block type II is better than safety profile of the thoracic paravertebral.

CONCLUSIONS

Pectoral nerve block type II provides a comparable to single-level thoracic paravertebral block analgesia efficacy during and after breast cancer surgery, and has a lower complications rate. These results allow consider this block

as a good alternative to TPVB. Further larger studies are required to confirm these findings by reaching statistically significant evidences and to investigate the Pecs II effects on the chronic pain development during the long-term period.

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ORIGINAL ARTICLE

AGE AND GENDER FEATURES OF DEPRESSIVE AND ANXIETY SYMPTOMATICS OF DEPRESSIVE DISORDERS

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ABSTRACT

The aim: of the study is to study the features of the clinical phenomenology of depressive disorders at the present stage, taking into account gender and age factors.

Materials and methods: Clinically examined 107 men and 138 women with bipolar affective disorder (current episode of depression), depressive episode and recurrent depressive disorder using HDRS, HARS, BDI, C. Spilberger's Scale of Reactive and Personality Anxiety.

Results: In all patients showed an increase in the severity of depression and anxiety with age, but in women the greatest differences were found between the middle and older age groups, and in men when comparing young and middle age groups. In young patients the level of agitated depression is higher for men, and women showed the higher overall level of anxiety, somatic anxiety, undifferentiated depression, reactive and personality anxiety. In patients aged 30-44, men have a significantly higher overall score of HARS depression, the level of adynamic, agitated and undifferentiated depression, the level of depression on BDI. In the older age group, the levels of depression in men and women are not significantly different, and the levels of mental anxiety and personality anxiety in women are significantly higher.

Conclusions: The general tendency consisting in increasing the severity of depression with age, and more pronounced indicators of depression in men, and anxiety in women. In men, the greatest increase in the severity of depression occurs before the age of 44 years, and in women after 45 years.

KEY WORDS: depressive disorders, depressive and anxious phenomenology

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INTRODUCTION

The problem of depression is one of the main problems of modern psychiatric science and practice; depressive disorders are associated with suicidal behavior, reduced of quality of life, and social disadaptation both in patients with depressions and in their microsocial environment [1-3]. According to modern studies, depressive disorders are characterized by polymorphism of symptoms, difficulty of diagnosis and resistance to therapy [4-7]. In this regard, it is important to study the clinical phenomenology of depressive disorders at the present stage, which can be the basis for the development of effective preventive measures [8-10].

THE AIM

The aim of the study is to study the features of the clinical phenomenology of depressive disorders at the present stage, taking into account gender and age factors.

MATERIALS AND METHODS

With the observance of the principles of biomedical ethics, we have clinically examined 107 men and 138 women who applied for medical care at Vinnitsa Regional Psycho-Neurological Hospital from 2015 to 2019. The nosological structure of the contingent was as follows: bipolar affective disorder, the current episode of depression (ICD-10 codes F31.3, F31.4): 42 men (39.8% of the total number

of men surveyed) and 38 women (27.5% of the total the number of women); depressive episode (F32.0, F32.1, F32.2): 32 men (29.9%) and 64 women (46.4%), recurrent depressive disorder (F33.0, F33.1, F33.2): 33 men (30.8%) and 36 women (26.1%), respectively.

The average age of the men was 34.2 ± 11.1 years, and 33.2 ± 11.4 years for women ($p=0.422$), the average duration of depression was 5.7 ± 5.8 years for men, and 4.5 ± 5.4 years for women ($p=0.064$).

3 subgroups were allocated in group of men and women depending on the age of patients at the time of the study: up to 30 years (M1 and W1, respectively), from 33 to 44 years (M2 and W2 groups, respectively), 45 years and older (M3 and W3 groups, respectively).

The study was conducted using M. Hamilton's Depression [11] and Anxiety Scale [12] (HDRS and HARS), A. Beck's Depression Inventory (BDI) [13], C. Spilberger's Scale of Reactive and Personality Anxiety in Y. Khanin modification [14].

Statistical analysis of differences between groups was carried out using non-parametric Mann-Whitney test.

RESULTS AND DISCUSSION

The results of the analysis of depressive and anxious manifestations in men are given in table I.

The main trend is an increase in the severity of depressive and anxious symptoms with age. This naturally reflects the

Table I. Features of depressive and anxious manifestations in men, taking into account age factor

Indicators	Clinical groups			level of statistical significance of differences (p)		
	M1 M±m/Me (Q ₂₅ -Q ₇₅)	M2 M±m/Me (Q ₂₅ -Q ₇₅)	M3 M±m/Me (Q ₂₅ -Q ₇₅)	M1 vs M2	M1 vs M3	M2 vs M3
M. Hamilton's Depression Scale (HDRS)						
Total score	15,8±5,8/13,5 (12,0-19,0)	22,3±9,1/21,0 (13,0-31,0)	28,7±11,1/33,0 (14,0-38,0)	0,002	0,000	0,010
Adynamic depression	11,8±4,7/10,0 (8,0-14,0)	16,0±6,6/16,0 (10,0-23,0)	21,2±7,1/24,0 (11,0-26,0)	0,003	0,000	0,003
Agitation depression	6,8±3,3/6,0 (4,0-10,0)	9,7±4,7/10,0 (6,0-14,0)	12,2±6,3/14,0 (4,0-17,0)	0,005	0,003	0,052
Depression with fear	5,7±4,4/4,0 (3,0-9,0)	8,5±5,1/8,0 (3,0-13,0)	12,4±5,9/15,0 (5,0-17,0)	0,014	0,000	0,005
Undifferentiated depression	3,0±2,0/2,5 (2,0-3,0)	5,4±2,5/5,5 (3,0-8,0)	6,0±3,0/7,0 (2,0-8,0)	0,000	0,001	0,288
M. Hamilton's Anxiety Scale (HARS)						
Total score	10,3±5,28,5 (7,0-11,0)	15,4±7,2/14,0 (10,0-22,0)	21,9±11,2/26,0 (8,0-33,0)	0,001	0,001	0,018
Psychical anxiety	8,3±2,5/8,0 (7,0-9,0)	10,2±3,4/9,5 (7,0-13,0)	11,9±4,6/14,0 (7,0-16,0)	0,011	0,006	0,130
Somatic anxiety	2,1±3,0/1,0 (0,0-3,0)	5,2±4,3/5,0 (2,0-8,0)	10,0±7,1/11,0 (1,0-16,0)	0,000	0,000	0,011
A. Beck's Depression Inventory (BDI)						
Total score	25,1±13,3/23,0 (15,0-34,0)	36,1±17,2/37,0 (19,0-53,0)	48,8±17,9/59,0 (26,0-62,0)	0,003	0,000	0,003
Cognitive-affective subscale	16,2±9,1/13,0 (10,0-24,0)	23,3±11,4/24,5 (12,0-34,0)	30,2±11,7/37,0 (13,0-39,0)	0,006	0,000	0,007
Subscale of somatic depressive manifestations	8,8±4,9/9,0 (5,0-12,0)	12,7±6,5/12,0 (7,0-19,0)	18,7±6,5/21,0 (13,0-23,0)	0,007	0,000	0,001
C. Spilberger's Scale of Reactive and Personality Anxiety						
Reactive anxiety	32,0±7,9/29,5 (26,0-32,0)	40,4±10,3/42,0 (32,0-49,0)	48,2±14,5/55,0 (29,0-62,0)	0,000	0,000	0,014
Personality anxiety	36,3±7,9/34,0 (30,0-40,0)	42,2±9,6/43,0 (33,0-51,0)	38,7±11,2/33,0 (31,0-47,0)	0,006	0,754	0,129

progression of the pathological process in time. However, the most pronounced differences in men were found when comparing groups up to 30 years and 30-44 years, while differences between groups of 30-44 years and 45 years and older are less pronounced.

For women, there are other patterns (Table II). They also showed an increase in the severity of depression and anxiety with age, but the greatest differences were found between the middle and older age groups. So, although the rates of depression in the middle age group are greater than in the young one, these differences are not statistically significant. Significant differences were found only for anxiety indicators (a general indicator for HARS, an indicator of somatic anxiety and an indicator of reactive anxiety). At the same time, in the older age group, all indicators are significantly more than in the middle.

Statistically significant differences between men and women were identified mainly in patients aged 30-44 (Table III).

In young patients under the age of 30 years the level of agitated depression is higher for men, and for women, the overall level of anxiety, the level of somatic anxiety and the level of reactive anxiety are higher. Also, women showed higher levels of undifferentiated depression and personality anxiety, but the level of statistical significance for these indicators is somewhat less. In patients aged 30-44, men have a significantly higher overall score of HARS depression, the level of adynamic and agitated depression and undifferentiated depression, the level of depression on BDI, including the cognitive-affective subscale and subscale of somatic manifestations of depression. In the older age group (45 years and more), the levels of depression in men and women are not significantly different, and the levels of mental anxiety and personality anxiety in women are significantly higher.

The study revealed some trends of depressive and anxious manifestations of depressive disorders in different age groups.

Table II. Features of depressive and anxious manifestations in women, taking into account age factor

Indicators	Clinical groups			level of statistical significance of differences (p)		
	W1 M±m/Me (Q ₂₅ -Q ₇₅)	W2 M±m/Me (Q ₂₅ -Q ₇₅)	W3 M±m/Me (Q ₂₅ -Q ₇₅)	W1 vs W2	W1 vs W3	W2 vs W3
M. Hamilton's Depression Scale (HDRS)						
Total score	14,6±6,1/12,0 (11,0-17,0)	18,0±9,0/14,0 (12,0-27,0)	28,1±10,3/31,0 (19,0-37,0)	0,076	0,000	0,000
Adynamic depression	10,8±4,6/9,0 (8,0-12,0)	12,9±6,7/10,0 (8,0-20,0)	20,1±6,8/22,0 (15,0-26,0)	0,189	0,000	0,000
Agitation depression	5,7±3,6/4,0 (3,0-8,0)	7,3±5,0/6,0 (3,0-12,0)	12,6±5,4/13,0 (8,0-17,0)	0,119	0,000	0,000
Depression with fear	6,5±4,0/5,0 (4,0-9,0)	7,7±5,7/5,0 (3,0-14,0)	12,6±5,6/14,0 (11,0-17,0)	0,693	0,000	0,001
Undifferentiated depression	3,5±1,9/3,0 (2,0-4,0)	4,4±2,6/3,0 (2,0-7,0)	6,4±2,8/8,0 (5,0-8,0)	0,318	0,000	0,002
M. Hamilton's Anxiety Scale (HARS)						
Total score	13,2±5,5/12,0 (9,0-17,0)	16,7±7,9/14,0 (11,0-23,0)	25,9±11,2/26,5 (15,0-36,0)	0,023	0,000	0,000
Psychical anxiety	9,5±4,0/8,0 (6,0-13,0)	10,8±4,4/10,0 (7,0-15,0)	15,0±5,8/15,5 (10,0-20,0)	0,074	0,000	0,001
Somatic anxiety	3,8±2,5/4,0 (2,0-5,0)	5,8±4,0/5,0 (3,0-8,0)	10,8±6,2/12,5 (6,0-16,0)	0,004	0,000	0,000
A. Beck's Depression Inventory (BDI)						
Total score	21,8±13,5/19,0 (11,0-31,0)	27,6±17,7/24,0 (14,0-45,0)	46,3±17,5/50,5 (35,0-61,0)	0,151	0,000	0,000
Cognitive-affective subscale	14,2±8,9/12,0 (6,0-21,0)	17,6±11,5/16,0 (7,0-28,0)	28,9±12,3/32,5 (19,0-39,0)	0,202	0,000	0,000
Subscale of somatic depressive manifestations	7,6±5,1/7,0 (3,0-11,0)	10,0±6,9/9,0 (4,0-15,0)	17,3±5,8/19,0 (13,0-22,0)	0,104	0,000	0,000
C. Spilberger's Scale of Reactive and Personality Anxiety						
Reactive anxiety	37,0±8,5/33,0 (31,0-44,0)	42,5±10,4/42,0 (33,0-50,0)	53,0±14,0/54,5 (42,0-65,0)	0,007	0,000	0,000
Personality anxiety	40,2±9,9/39,0 (31,0-44,0)	40,3±8,5/41,0 (33,0-44,0)	49,7±10,9/54,0 (42,0-58,0)	0,725	0,000	0,000

It has been established that, in general, men have higher rates of depression than women. This may be due to the fact that men seek psychiatric help later, and in more severe cases than women because of the stereotype that exists in society that a man must be enduring and endure difficulties. Perhaps men are also more affected by stigmatization. In women, higher levels of anxiety were revealed, which can be considered as one of the manifestations of gender pathomorphosis of depression.

It has been established that, in general, the severity of depressive and anxious manifestations in depressive disorders increases with age. However, in men and women, the trends are different: in men, a more pronounced deterioration was observed before the age 44 years, and in women – after 45 years. These features require further deeper study of the dynamics and pathomorphosis of depressive disorders, taking into account gender and age factors.

CONCLUSIONS

The study of the features of depressive and anxious manifestations of depressive disorders revealed its main tendencies, consisting in increasing the severity of depression with age, and more pronounced indicators of depression in men, and anxiety in women. In men, the greatest increase in the severity of depression occurs before the age of 44 years, and in women after 45 years.

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Table III. The levels of statistical significance of differences when comparing men and women

Indicators	Level of statistical significance (p)		
	M1 vs W1	M2 vs W2	M3 vs W3
Total score on HDRS	0,137	0,012	0,744
Adynamic depression subscale of HDRS	0,197	0,015	0,376
Agitation depression subscale of HDRS	0,047	0,016	0,851
Depression with fear subscale of HDRS	0,154	0,394	0,954
Undifferentiated depression subscale of HDRS	0,061	0,048	0,519
Total score on HARS	0,001	0,435	0,213
Psychical anxiety on HARS	0,371	0,627	0,033
Somatic anxiety on HARS	0,000	0,367	0,702
Total score on BDI	0,222	0,015	0,479
Cognitive-affective subscale of BDI	0,248	0,014	0,788
Subscale of somatic depressive manifestations of BDI	0,185	0,040	0,324
Reactive anxiety on C. Spilberger's Scale	0,001	0,399	0,278
Personality anxiety on C. Spilberger's Scale	0,080	0,351	0,001

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ORIGINAL ARTICLE

A NEW PERSPECTIVE ON BRONCHIAL ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE OVERLAP: POTENTIAL DIAGNOSTIC CRITERIA

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ABSTRACT**The aim:** The aim of the research was to analyze the results of observation and examination of COPD patients in order to identify a group of individuals with potential asthma overlap.**Materials and methods:** We have conducted a two-stage dynamic investigation of 43 COPD patients during 3–8 years. The patients were divided into two groups: group 1 counted 30 individuals who presented with at least one episode of reversible bronchial obstruction (RBO) during the observation; group 2 – 13 individuals who presented with nonreversible bronchial obstruction (nonRBO). At the first stage, we conducted a clinical observations analysis and studied lung function examination records; at the second stage, we calculated the markers of allergic inflammation.**Results:** It was revealed that around 70% of COPD patients have occasional episodes of RBO. It was established that the level of blood eosinophils in these patients on the whole is rather low even in people with intermittent RBO, and the total IgE level appeared to be significantly higher in patients with intermittent RBO comparing to the level of this marker in patients who have nonRBO.**Conclusions:** COPD patients with intermittent RBO and high level of total IgE level form a group with potential asthma overlap.**KEY WORDS:** COPD, asthma, overlap, allergic airway inflammation, bronchial obstruction

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INTRODUCTION

In the late 20th – early 21st century, a lot of academic research dealt with different issues related both to asthma [1, 2], and to chronic obstructive pulmonary disease (COPD) [3, 4, 5].

In 2014, after an extensive review of available academic literature, international experts first discussed in GINA and GOLD guidelines the possibility of asthma combination with COPD [6]. Later on, this combination was referred to as ACO, i.e. Asthma-COPD overlap [7]. To date, this topic is being actively discussed seeking to elaborate standards both for diagnosis, and for patient treatment. This includes updating diagnostic criteria for ACO, identifying diagnostic significance of the markers of pathologies combination at different stages of disease course, developing indications for medication administration at certain stages of disease course, specifying medication dose, etc. [8, 9, 10].

In our opinion, the combination of the two aforementioned diseases may progress in different ways: the first clinical case is a COPD overlap in the course of the existing asthma; the second case is an asthma overlap in the course of the existing COPD.

The first clinical case has been studied in more detail. It is shown that among patients with bronchial asthma, particularly among smokers, there are individuals with predominantly neutrophilic inflammation, rapid decrease in pulmonary ventilation or poorer response to bronchodilator or inhaled

glucocorticosteroid (ICS) therapy [6, 7, 8]. It is also believed that the possibility of ACO formation in patients with asthma may be associated with risk factors for constantly progressing airflow limitation, such as childhood asthma, long-term asthma with no ICS intake, first asthma manifestations in adulthood, severe or treatment-resistant asthma [8, 9, 10]. According to the latest findings, ACO formation in patients with previously verified asthma may reach 29% [9].

The second clinical case is quite rarely discussed in academic literature. Yet, it has been determined that a significant number of patients with COPD may occasionally present with reversible bronchial obstruction (RBO) [10, 11], which is more typical of asthma. In COPD, it is mainly explained by bronchial hyperresponsiveness (one of its mechanisms is an expression of a CD38 marker, which catalyzes the formation in smooth muscle cells of cyclic ADP-ribose with further calcium mobilization from intracellular stores [12]) or insufficient or inadequate pharmaceutical treatment [13]. It is also found that, among COPD patients, certain individuals' laboratory test results are more typical of asthma, which is particularly true for high sputum eosinophil count [11]. To date, the mechanisms of connection between clinico-functional and laboratory findings in ACO haven't been clearly identified; the criteria for unified clinical diagnosis verification have not been determined, either. In view of this, our research focused on this very issue.

THE AIM

The aim of the research was to analyze the results of long-term observation and examination of patients with previously verified COPD in order to identify a group of individuals with possible asthma overlap as well as to determine the markers of COPD and asthma combination.

MATERIALS AND METHODS

The research was carried out in two stages. At the first stage, we analyzed conducted a clinical observations analysis and studied the records of dynamic lung function examination of 43 COPD patients in stable condition only. The observation period made 3–8 years, during which 240 clinic visits were performed (an average number of visits per each patient was 6.5 (2.2)). At all visits, the severity of ventilatory failure was determined in all patients via spirometry with the calculation of forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) in absolute and relative values; the degree of airway obstruction reversibility was determined via bronchodilator testing (400 mcg salbutamol delivered by a spacer device). Based on post-bronchodilator FEV_1 , the patients were referred to groups GOLD II and III.

At the second stage of the research, allergic status markers were identified for all the aforementioned patients: we performed a complete blood count to calculate eosinophil level in absolute value (μL) and measured the total serum IgE (IU/ml) using the enzyme-linked immunosorbent assay.

The COPD diagnoses were formulated according to the Order of the Ministry of Healthcare of Ukraine № 555 from 27 June 2013. All patients received adequate background therapy depending on the clinical group of the disease and according to international and national guidelines depending on the severity of disease course (long-acting bronchodilators with/without ICS as a routine therapy and short-acting β_2 -agonist salbutamol as required).

All patients gave a written consent for retrospective clinical observations analysis and laboratory investigations.

The study was conducted according to Helsinki declaration with the permission of the Bioethical commission Dnipropetrovsk medical academy of the Ministry of Health of Ukraine.

Statistical data processing were performed using the STATISTICA 6.1 software ("StatSoftInc", serial number AGAR909E-415822FA). Quantitative data were analyzed after the arithmetic mean and standard deviation at normal markers distribution and after the median and interquartile range [25%;75%] (in M (SD)) at non-normal markers distribution. The type of quantitative data distribution was determined after the Shapiro-Wilk (W) and Kolmogorov-Smirnov (D) criteria; the data were clinically significant at $p > 0.05$. Qualitative characteristics of the markers (elevated/normal) were presented as a number of patients (n) having those markers, and as relative frequencies expressed as a percentage (%). To compare two independent groups of markers, we used the Mann-Whitney U-test (U) in case of non-normal data distribution. The probability of differences in the relative indices (of data distribution) was identified via Fisher's exact test; the differences between the groups at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The clinical observations analysis at the end of the first stage of the research showed that male patients were a majority (35 (81.4%)); an average age at the first clinic visit was 62.0 (7.15); body mass index (BMI) was 27.3 (4.3); all patients had a history of smoking in the past or upon initial examination (a pack-year ratio – 27.8 (4.9)); disease duration – 15.7 (3.9) years.

We noticed that at the initial stages of COPD development, the patients' diagnosis was clear: the patients complained of first clinical signs of the disease at the age > 40 (at first, they presented primarily with a cough; further on, they developed a shortness of breath, increased cough, sputum containing mucus and pus); the presence of a post-bronchodilator $FEV_1/FVC < 0.70$ was confirmed spirometrically.

At patients observation stages (during each subsequent clinic visit), we controlled both clinical, and spirometrical markers. It was established that a certain part of the patients had developed clinical symptoms variability – at different visits, shortness of breath and cough had different severity, despite the fact that the patients received regular and full-fledged medication therapy. Besides, the same patients appeared to have variable spirometrical markers. At the same time, we encountered with the patients whose clinical symptoms and functional performance remained stable throughout the long-term observation. Following the above mentioned, all examined patients were divided into two groups: group 1 counted 30 (69.8%) individuals who presented with at least one episode of RBO during the long-term observation (post-bronchodilator FEV_1 increased by $> 12\%$ and > 200 ml comparing to baseline characteristics; FEV_1 -increase fluctuation had a wide range of 12.2 to 69% and of 230 to 910 ml); group 2 counted 13 (30.2%) individuals who presented with nonreversible bronchial obstruction (nonRBO) throughout the whole observation period. The average age in group 1 didn't differ from that in group 2 (60.6 (7.18) and 65.2 (6.19) years, respectively) (two-tailed Student's t-test: $t = 1.83$; the number of degrees of freedom is 41; $p = 0.074$); body mass index (BMI) was similar in both groups (27.5 (4.5) and 26.8 (3.8) kg/m^2 , respectively) (two-tailed Student's t-test: $t = 0.46$; the number of degrees of freedom is 41; $p = 0.647$). Thus, it was demonstrated that around 2/3 of patients with previously verified COPD have occasional episodes of RBO. Obviously, RBO may be associated with a number of factors. However, in order to exclude the possibility of asthma overlap in these patients, the following stage of the research was performed.

At the second stage, it was shown that the identified groups of patients had somewhat different number of individuals with elevated and normal blood eosinophils and/or total IgE (Table 1).

It is noteworthy that, if group 1 presented with elevated eosinophil count a bit more frequently than group 2 (although statistically inaccurate), elevated total IgE was detected in group 1 in 40% of cases, while in group 2 it was not detected in any case. Consequently, the eosinophil marker demon-

Table 1. Patient distribution according to blood eosinophils and total IgE

№	Markers	Groups		p
		1, abs (%)	2, abs (%)	
1.	Blood eosinophils:			> 0.05
	elevated (over 0.5 µL)	4 (13.3)	1 (7.7)	
	normal	26 (86.7)	12 (82.3)	
2.	Total IgE:			< 0.05
	elevated (over 100 IU/ml)	12 (40.0)	0 (0)	
	normal	18 (60.0)	13 (100)	
3.	At least one of the aforementioned markers:			< 0.05
	elevated	14 (46.7)	1 (7.7)	
	normal	16 (53.3)	12 (82.3)	

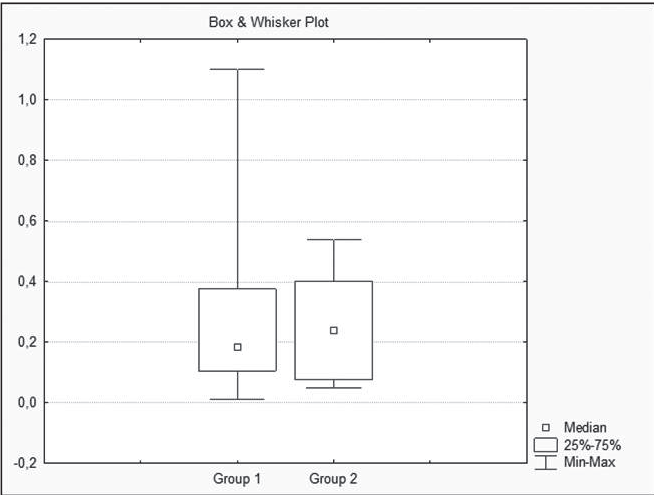


Fig 1. Eosinophils levels in the groups of examined COPD patients

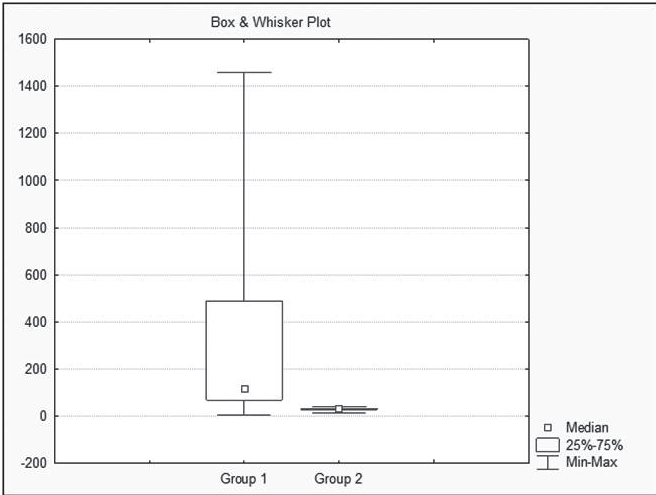


Fig 2. Total IgE in the groups of examined COPD patients

strated a quite low sensitivity as to the identification of the patients' allergic state, since in many of those, at high total IgE, blood eosinophils didn't exceed the reference values.

The absolute values of allergic state markers in the groups of the examined patients are provided in Fig 1 and 2.

It was discovered that blood eosinophils in COPD patients in the stable disease phase are in general quite low, and in the majority of individuals (86.1%) they do not exceed 0.4 µL. Besides, this marker in patients with COPD who have intermittent RBO (group 1) doesn't differ from that in patients who have nonRBO (group 2) (p=0.827).

Total IgE in COPD patients who have intermittent RBO appeared to be significantly higher than that in patients who have nonRBO (p=0.008).

Our findings match the results of other researches, which show that peripheral blood eosinophil count in COPD patients doesn't correlate either with bronchial hyperresponsiveness rate, or with the number of disease exacerbations, and is in general quite low [13, 14, 15].

Our findings don't contradict the recent study, which also demonstrates that increased total IgE (44%) is pretty common for COPD patients. The latter may witness to the lower airway allergic inflammation in certain COPD patients even without any clinical manifestations of atopy, and therefore, be a marker of ACO [15].

Therefore, our research enabled us to identify a group of COPD patients, whose diagnosis at the initial stages of the disease was clear and characterized by the following typical signs: long-term tobacco smoking, first clinical manifestations of the disease at the age > 40, gradually increasing shortness of breath and cough with sputum containing mucus and pus, spirometrically confirmed the presence of a post-bronchodilator FEV1/FVC < 0.70. However, with the diseases development, these patients presented with the signs of a different illness – asthma: intermittent RBO, elevated total IgE and sometimes blood eosinophilia. Keeping in mind a stage-like nature of diseases overlap, we recommend referring to such clinical cases as CAO (i.e. COPD-Asthma overlap) instead of ACO. The formation of CAO may require patient treatment revision and management; particularly, treatment regimen should include high doses of ICS if patients have not taken any before.

CONCLUSIONS

- 1. COPD patients require compulsory long-term dynamic observation not only to control clinical and functional performance and medication therapy management (as appropriate), but also to exclude possible asthma overlap with further CAO development and, consequently, to manage treatment strategy.

2. Blood eosinophilia determination in COPD patients is not recommended for identifying the possibility of CAO formation due to a low diagnostic sensitivity of this marker.
3. Both dynamic control of RBO and total serum IgE determination (at least occasional) are recommended for verifying CAO formation in the course of COPD.
4. In case of CAO formation, patient follow-up should lean on diagnostic and treatment standards not only for COPD, but also for bronchial asthma.

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ORIGINAL ARTICLE

STUDY OF ANTI-VIRUS ACTIONS OF METABOLITES OF LACTOBACTERIA

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ABSTRACT

The aim: of the work was to study the antiviral activity of the metabolites of the probiotic strain *Lactobacillus rhamnosus* GG (LGG or ATCC 53103) regarding clinical strains of enteroviruses (Coxsackie B-5, ECNO21) isolated from the feces of intestinal infections.

Materials and methods: The object of the study was substrate-dependent cell cultures of HeLa, Vero, Hep-2 lines. The titer of the virus was determined by the presence of a clear cytopathic action (CPA) in the monolayer infected cells of the virus.

Results: Determination of the enteric virus infections activity in the culture fluid showed that in samples with the LGG metabolites, the infections activity of the clinical strains of enteroviruses decreased after 24 hours, at 1.5-1.7 ($p < 0.05$) times, and after 96 hours in 3, 6 – 5,7 times ($p < 0.01$). the processing of cell cultures by metabolites in the amount of 0.3 mg / ml contributed to a decrease in the titer of viruses by 2.77 ± 0.11 lg TCDD50 / cm³, 2.83 ± 0.11 lg TCD50 / cm³ and 2.94 ± 0.13 lg TCD50 / cm³ for Vero, HeLa and Hep-2 line cells in 24 hours.

Conclusions: It has been experimentally determined that the maximum tolerated dose (MTD) of *L. rhamnosus* GG metabolites was 0.3 µg / ml for all cultures of cell lines. Determination of the antiviral activity of *L. rhamnosus* GG metabolites in clinical viruses of enteroviruses (Coxsackie B-5 and ECNO-21) showed a decrease in infection activity in 1.5-1.7 times, ($p < 0.05$) of clinical trials in clinical trials enteroviruses.

KEY WORDS: probiotic, cell cultures, antiviral activity, metabolites

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INTRODUCTION

In recent years, the link between human microbiocenosis and the state of its immune system is an important direction for the research of Ukrainian and foreign scientists [1 – 5]. Special attention of researchers attracts such representatives of normal flora as *Lactobacterium* and *Bifidobacteria* and probiotic drugs on their basis. It is known that course application of oral probiotics containing lactobacilli, helps to increase the resistance of the human body to diseases not only bacterial but also viral etiology [6,7].

Today, probiotic drugs are considered to be the most promising means against many pathogens, since the leading role of normobiots of the human body in the preservation of its health has been proven. It is known that the anti-infective effect of probiotic drugs is due, first of all, to the antagonistic relationship between the probiotic strain and the pathogenic agent. Antagonism of probiotic strains is ensured by the production of biologically active substances (metabolites) that inhibit the development of pathogens [8].

As for the study of antiviral activity of probiotic drugs, research into such a plan requires an adequate model. To study

the antiviral activity of probiotic drugs as a model for preclinical testing of drugs, biologically active substances, etc., the actual direction is the use of cell cultures, because they are able to specifically respond to the influence of biotic factors. Specificity, as a rule, manifests itself in the form of a cytotoxic effect, which is evaluated by the degree of morphological changes, morphological disorders and proliferative properties of cells.

THE AIM

The purpose of the work was to investigate the antiviral activity of metabolites of the probiotic strain *Lactobacillus rhamnosus* GG (LGG or ATCC 53103) on the clinical strains of enteroviruses (Coxsackie B-5, ECNO21) isolated from feces in patients with intestinal infections

MATERIALS AND METHODS

The object of the research was substrate-dependent HeLa, Vero, Hep-2 cell strains. They are morphologically epithelial cells by nature.



Figure 1. Skin reaction at the place of metabolite injection
Notations: A – Hyperemia; B – Hyperemia and infiltrate.

VIROLOGICAL METHODS

Virus titer was evaluated based on the occurrence of cytopathic action (CPA) in the cells of culture monolayer such as appearance of round-shaped cells, detachment of the majority of the cells from glass surface and monolayer disruption. The titration of virus according to its CPA was based on determination of the largest dilution of the material contained the virus that could cause CPA in 50% of infected cell culture (TCA_{50}). Intensity of CPA was assessed by «plus system» (4+). Determination of TCA_{50} in the volume of the material taken for the single monolayer of cell culture was accomplished by Cerber's method. The Creber's equation for virus titer can be outlined in the following way:

$lg TCA_{50} = L - d (S-0,5)$, where

L – lg of the least dilution used during titration;

D – the interval of lg between two consecutive dilutions;

S – the sum of the proportions of positive test-units (namely, flasks with culture where CPA took place) at each dilution.

Antiviral activity was investigated using Vero, HeLa and Hep-2 strains. Their seeding concentration was $0,5 \times 10^6$ /ml. The monolayer of these cells was treated with metabolites of LGG with concentrations of 0.3, 0.2, 0.1 mg/ml. Additionally, strains of Cocksackie B-5 and ECNO-21 viruses were introduced after 24 hours. Cytopathic action on cell state was checked every 24 hours with inverted microscope. Infectious activity titer was evaluated in experimental and control samples after 96 hours.

MICROBIOLOGICAL METHODS OF INVESTIGATION

Cell-free filtrates of cultural media obtained during *L.rhamnosus* GG cultivation were used as metabolites. The cell suspension of 24-hour *L.rhamnosus* GG culture with optical density of 1.0 McF was used for preparation of these metabolites. 5 ml of the prepared cell suspension were added to 45 ml of nutritional broth with 1% glucose. Then the mixture was incubated for 72 hours at 37°C under microairlift conditions. After that it was centrifuge at 3000 cycle/min (centrifuge ОПН-3 У 42, 1200G). for 30 minutes. The supernatant was collected and filtered through sterile net of 0.2 μ m in diameter (Vladipor, Russia). The procedure was repeated 3 times. These metabolites were checked for sterility.

BIOCHEMICAL METHODS

The level of protein nitrogen was determined in accordance with “The determination of protein nitrogen with Nessler's reagent by preliminary precipitation of the protein material in immunobiological drugs”.

The method was based on the ability of Nessler's reagent to confer coloration during its reaction with free ammonia released after mineralization of protein containing products. 1 ml of metabolites in centrifuge tube was added with 1 ml of 20% trichloroacetic acid (TCA), carefully shaken and left for 18-20 hours at 4-8°C. Then the samples were centrifuged for 30 min at 2000 cycles/min (centrifuge ОПН-3 У 42, 600G). The pellet was washed with 1 ml of 10% TCA. The centrifugation was repeated. After that 0.1 ml of concentrated H_2SO_4 was added to the pellet. The samples were incubated in sandy bath for 10 hours at 190-200°C. A sample that contained only H_2SO_4 was used as a control sample. Then 9.9 ml of distilled water was added to the mineralizate. After that 0.5 ml of mineralizate was taken in case of sample with expected content of PNU of about 0.08 to 0.2 mg/ml, diluted with distilled water to 9.5 ml and carefully mixed. Then 0.5 ml of Nessler's reagent was added and mixed again. In case of the sample with expected content of PNU of about 0.2 to 0.4 mg/ml up to 1 ml of mineralizate was used. Metabolites of *Lactobacilli* were tested for PNU for standardization. Their nitrogen content was adjusted to 0.1-0.3 mg/ml. These doses were chosen to study sensitizing properties in laboratory animals (rabbits weighing from 1.5 to 2.0 kg)

The optical densities of all samples were measured at the wavelength of 400 nm against control. Cuvettes' width was 10 mm. The calibration curve was drawn according to commercial standard sample of “Protein nitrogen content” (Pharmacea, Russia). The amount of protein nitrogen was calculated using equation below:

$$D = \frac{a \times 10}{A \times B \times 1000}, \text{ where}$$

X – PNU;

a – the amount of nitrogen according to calibration curve, μ g;

10 – mineralizate dilution;

A – the volume of investigated sample;

B – the volume of mineralizate, ml;

1000 – the transition of μ g to mg;

Table I. The degree of cell monolayer degeneration under the cell cultivation in growth medium supplemented with *L. rhamnosus* GG metabolites.

Cells lines	Time observation, an hour	Amount of metabolites, mg / ml PNU						
		0,3	0,2	0,1	0,05	0,025	0,0125	0,00625
HeLa	24	-	-	-	-	-	-	-
	48	+	+	±	-	-	-	-
	72	++	++	++	±	-	-	-
Vero	24	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	-
	72	±	-	-	-	-	-	-
Hep-2	24	±	±	-	-	-	-	-
	48	+++	++	+	+	±	-	-
	72	++++	+++	++	++	+	±	-

STATISTICAL METHODS

Each experiment of the research was repeated 3 to 5 times. Statistical processing of data were carried out in accordance with the rules of the ordinary and alternative variation statistics.

RESULTS AND DISCUSSION

In the study of sensitizing properties of metabolites of lactobacilli in laboratory animals a dose of 0.1 mg/ml caused a questionable local allergic reaction (hyperemia of the skin), and a dose of 0.3 mg/ml caused a slightly positive reaction (the appearance of a blister with skin hyperemia around it) (Figure 1). Higher concentration of metabolites may cause necrosis at the place of injection meaning that such doses should be avoided.

The changes in morphology of cell culture (HeLa, Vero, Hep-2 were used) were used to assess cytotoxic action of probiotic strains.

First, the effect of sugar broth used for isolation of metabolites on adhesive properties and on the presence or absence of alterations in the monolayer of HeLa, Vero, Hep-2 cell cultures was investigated. It was shown that the addition of 0.00625 to 0.5 ml of sugar broth to 1 ml of growth media did not alter the adhesive properties of HeLa, Vero, Hep-2 strains and monolayers' integrity.

The 24-hour action of probiotic *L. rhamnosus* GG metabolites on cell cultures resulted in no statistical change in HeLa, Vero, Hep-2 adhesiveness. After 48 and 72 hours of incubation all of the control cultures formed monolayers without any signs of degeneration. As far as the degree of degeneration of experimental cultures is concerned, the most stable to *Lactobacilli* metabolites were Vero cells. Only 72-hour incubation in growth medium supplemented with 0.3 mg/ml metabolite of agent of influence affected some cells of this line. This corroborated the insensibility of this culture to the agent of influence (Table I).

HeLa cells had moderate sensitivity to lactobacilli metabolites. Some lesions were traced in 25 % of these cells after 48-hour incubation in 1 ml of growth media supplemented with a dose of metabolites that contained 0.3 mg/ml and 0.2 mg/ml of the agent of influence. The addition of 0.1

mg/ml *L. rhamnosus* GG metabolites to growth medium resulted in the breakdown of some cells of this line after 48 hours of incubation. Lesions were seen in 50% of HeLa cells that were cultivated with 0.3, 0.2 and 0.1 mg/ml of metabolites after 72 hours. Some individual disrupted cells were found in growth media supplemented with 0.05 mg/ml of metabolites.

The most sensitive to *L. rhamnosus* GG metabolites were Hep-2 cells. Some damaged cells were observed after 24 hours of incubation with 0.3 and 0.2 mg/ml of metabolites. Even the addition of as little dose as 0.025 mg/ml had an effect. 100%, 75%, 50%, 50% and 25% of these cells were damaged after 72-hour incubation with 0.3, 0.2, 0.1, 0.05, and 0.025 mg/ml of *L. rhamnosus* GG metabolites, respectively. Moreover, some individual cells died in presence of 0.0125 mg/ml.

The next phase of the research was elicitation of antiviral activity of metabolites of probiotic strain of *L. rhamnosus* GG.

It was found that clinical strains of enteroviruses could cause destruction in 50% of experimental cells (HeLa, Vero, Hep-2) as early as 24 hours after the beginning of observation (Table II, III).

The first signs of enteroviral CPA (Cytopathological action) could be seen as HeLa cell breakdown after 48 hours of the HeLa monolayer treatment with 0.3 and 0.2 mg/ml PNU LGG metabolites. 50% of these cells were damaged after 72 hours with respect to 100% in control. 50% of damaged cells of HeLa strain were detected after 48 hours of the monolayer treatment with 0.1 mg/ml PNU LGG metabolites and clinical enteroviral strains.

The treatment of Vero strain with LGG metabolites in concentration of 0.3 mg/ml PNU resulted in appearance of the first signs of individual cell lesions after 48 and 72 hours under Coxsackie virus and ECNO-21 virus action, respectively. Lower doses of metabolites (0.2 and 0.1 mg/ml PNU) led to appearance of the enteroviral CPA after 24 hours of introduction.

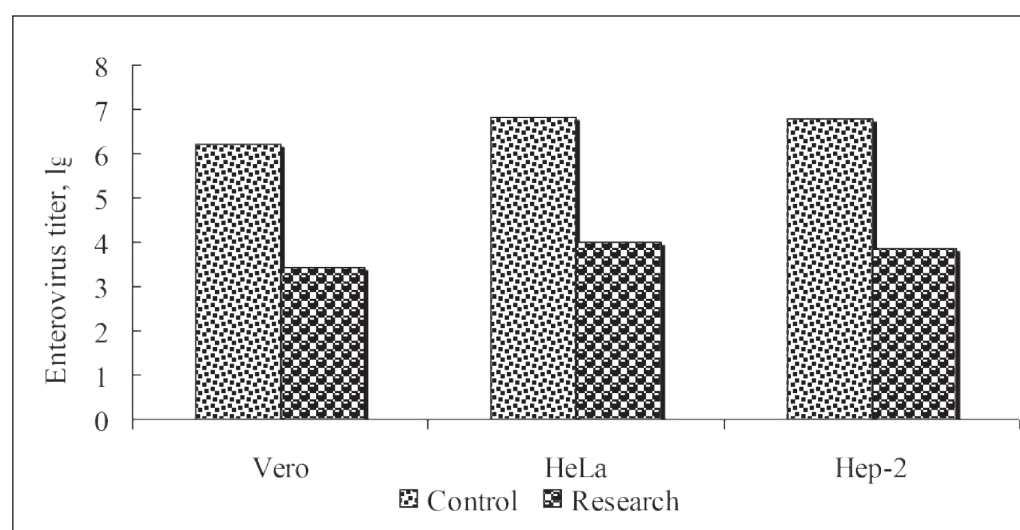
In the case of treatment of Hep-2 with LGG metabolites with 0.3 and 0.2 mg/ml PNU the first signs of enteroviral CPA manifested after 24-48 hours. After 78 hours the destruction was observed in 50 and 75% of cells under ECNO-21 and Coxsackie virus action, respectively, when in control all of the cells (100%) were damaged. The treatment

Table II. Cell status under cytopathic action in the presence of the metabolites of LGG and the clinical strain ECNO-21.

Cells lines	Time observation, an hour	Amount of metabolites, mg / ml PNU			
		K (ECNO-21)	0,3	0,2	0,1
HeLa	24	++	-	-	+
	48	+++	±	+	++
	72	++++	++	++	+++
Vero	24	++	-	+	+
	48	+++	-	++	++
	72	++++	±	+++	+++
Hep-2	24	++	+	+	++
	48	+++	++	++	+++
	72	++++	+++	+++	+++

Table III. Cell status under cytopathic action in the presence of the metabolites of LGG and the Coxsackie virus B-5.

Cells lines	Time observation, an hour	Amount of metabolites, mg / ml PNU			
		K (Coxsackie virus B-5)	0,3	0,2	0,1
HeLa	24	++	-	-	±
	48	+++	±	+	++
	72	++++	++	++	+++
Vero	24	++	-	+	+
	48	+++	±	++	++
	72	++++	±	+++	+++
Hep-2	24	++	±	±	+
	48	+++	+	+	++
	72	++++	++	++	++

**Figure 2.** Enterovirus titer in different cell lines after 24 hours of infection.

of Hep-2 strain with LGG metabolites that contain 0.1 mg/ml PNU resulted in the breakdown of 50 and 75% of these cells under Coxsackie B-5 and ECNO virus actions.

The investigation of enteroviral infectious activity in cultural media showed that samples supplemented with LGG metabolites after 24 hours had 1.5-1.7 times lower viral activity ($p < 0.05$). After 96 hours the viral activity

decreased 3.6-3.7 times ($P < 0.01$). The best results were obtained when maximum tolerated dose was used. The treatment of cell culture with metabolites that contained 0.3 mg/ml PNU favored virus titer decrease after 24 hours by 2.77 ± 0.11 lg TCA50/cm³, 2.83 ± 0.11 lg TCA50/cm³ and 2.94 ± 0.13 lg TCA50/cm in Vero, HeLa and Hep-2, respectively (Figure 2).

Thus, the protective and antiviral action of *Lactobacillus rhamnosus* GG metabolites has been represented with respect to HeLa, Vero, Hep-2 strains under Cocksackie and ECNO-21 viral infections.

Experimentally examined maximum tolerated dose (MTD) for all of these cultures came to 0.3 µg/ml. Inhibiting doses that decreased the amount of viable cell to 50% (ID₅₀) were equal to 0.6 µg/ml for Vero strain and 0.5 µg/ml for HeLa and Hep-2 strain.

Thus, it has been demonstrated that HeLa, Vero and Hep-2 possess different sensitivity to lactobacilli metabolite action. The most sensitive were HeLa and Hep-2. The effect was dose-dependent. These facts corroborate that these cell cultures can be used for the pre-clinical testing of metabiotic medications.

CONCLUSIONS

1. An estimation of the use of cell cultures (HeLa, Vero, Hep-2) for the study of the antiviral activity of the probiotic strain *Lactobacillus rhamnosus* GG (LGG or ATCC 53103) was performed. It has been experimentally determined that the maximum tolerated dose (MTD) of *L. rhamnosus* GG metabolites was 0.3 µg / ml for all cultures of cell lines.
2. Determination of the antiviral activity of *L. rhamnosus* GG metabolites in clinical viruses of enteroviruses (Cocksackie B-5 and ECNO-21) showed a decrease in infection activity in 1.5-1.7 times, (p < 0.05) of clinical trials in clinical trials enteroviruses. Thus, the antiviral activity of *L. rhamnosus* GG metabolites has been experimentally determined.

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ORIGINAL ARTICLE

FREQUENCY OF IMPLEMENTATION AND IMPACT OF APPENDECTOMY AT ACTIVATION OF THE PATHOLOGICAL PROCESS IN PATIENTS WITH CHRONIC NON-ATROPHIC GASTRITIS

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ABSTRACT

The aim: Determine the frequency of surgery-appendectomy of patients with chronic non-atrophic gastritis and the impact of this transaction on the pathogenesis of chronic gastritis.

Materials and methods: Data of disease history and life were analyses, as well as the results of a comprehensive survey of 245 patients with chronic non-atrophic gastritis. Comprehensive examination included: step-by-step pH-metry, esophagogastroduodenoscopy, helicobacter infection test (HP) (helicobacter urease test and microscopic examination of stained smears), histological investigations of the gastric stump mucous.

Results: Helicobacter infection was detected in 100% of cases. It was found that 56 (22.9%) of patients were subjected to appendectomy. Age of patients, who had an appendectomy ranged from 4 to 40 years and averaged 18.34 ± 1.05 years, and the first pathological manifestations of the gastro-intestinal tract appeared in an average of 28.27 ± 1.75 year, i.e. in 10 years. As for the age qualification pupil were the earliest pathological manifestations appeared in a group of patients from 11 to 15 years (13 people (23.2%) and amounted to about 6 years after the operation, and 6 (46.2%) patients, manifestations appeared in 2-6 months after surgery; the most recent is in group from 16 to 20 years (19 people (33.9%) and amounted to about 14 years ($p < 0.05$).

Conclusions: Surgery on the body of immune system – appendix provokes activation of latent form of chronic non-atrophic gastritis, especially during puberty.

KEY WORDS: chronic non-atrophic gastritis, the immune system of the human body, appendectomy

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INTRODUCTION

The condition of immune system is the key to curbing the development of pathological processes in the human body, so any intervention in its function is fraught with the development of different diseases. Appendicular process is part of the immune system, since it, like in the tonsils, formation of B-lymphocytes, which are the basis of humoral immunity [1, 2, 3, 4, 5, 6, 7, 8, 9]. Appendectomy remains the most common surgery: a year in the US, up to 250.000 appendectomies [10, 11, 12]. In the available for us literature there was no information on how appendectomy effects on the activation and development of chronic non-atrophic gastritis, which was the reason for our research.

THE AIM

Determine the frequency of surgery-appendectomy of patients with chronic non-atrophic gastritis and the impact of this transaction on the pathogenesis of chronic gastritis.

MATERIALS AND METHODS

On the basis of the clinical division of the problem lab on chronic Helicobacter pylori infection of the Petro Mohyla Black Sea National University data of disease history and life

were analyses, as well as the results of a comprehensive survey of 245 patients with chronic non-atrophic gastritis. The age of patients ranged from 25 to 61 years (the medium age was 38.3 ± 1.45); males (80.4%) were 197, female – 48 (19.6%).

Primary comprehensive survey included: step-by-step intragastric pH-Metry based on methodology by Chernobrovii V.N esophagogastroduodenoscopy(EGDS) by generally accepted method, double HP testing: test for ureaz activity and microscoping stained by Giemsa smears, material for which was taken during endoscopy from 4 topographical zones: from the middle third of the gastric antrum and body of stomach on the big and small curvature with our created methodology and also histological studies of the gastric mucosa, the material for which is taken from the same zone, using a generally accepted method taking into account recent classification [13].

The study was conducted in accordance with the basic bioethical provisions of the Helsinki Declaration of the World Medical Association on the ethical principles of scientific 549 medical research involving human (1964-2008) and the order of the Ministry of Health of Ukraine No. 690 dated September 23, 2009, which was confirmed by the findings of the meeting of the Ethical Commission of Petro Mohyla Black Sea National University, Nikolaev No. 5 dated May 15, 2019.

Table I. Level of acidity of the patients with chronic non-atrophic gastritis

The level of acidity	The number of surveyed patients with chronic non-atrophic gastritis (n = 245)	
	Number of patients	%
Hyperacidity expressed	24	9,8
Hyperacidity moderate	35	14,3
Normacidity	108	44,1
Hypoacidity moderate	47	19,2
Hypoacidity expressed	31	12,6
Anacidity	0	0

Note: n-the number of studies

Table II. Degree of semination of gastric mucosa with HP infection on topographical zones of patients with chronic non-atrophic gastritis

Topographic zones of stomach	Degree of semination of gastric mucosa with HP infection on topographical zones of stomach (+) / (M ± m) (n = 245)
Antrum of the stomach	a) 2,25 ± 0,20; 6) 2,18 ± 0,20.
Body of stomach	a) 2,15 ± 0,20; 6) 2,13 ± 0,20.

Note: n-the number of studies, a) large curvature, b) small curvature

Table III. Age of patients during appendectomy and beginning of the first pathological manifestations after surgery (n = 56)

Age qualification in the operation period	Number	%	M ± m (year)	First pathological manifestations M ± m (year)
Before 10 years	7	12,5	6,57 ± 0,83	17,29 ± 2,49
From 11 to 15 years	13	23,2	13,38 ± 0,31	19,77 ± 2,91
From 16 to 20 years	19	33,9	18,21 ± 0,25	32,58 ± 1,80
From 21 to 25 years	7	12,5	22,85 ± 0,49	30,57 ± 2,94
Older then 25 years	10	17,9	30,10 ± 1,34	37,20 ± 4,21

Note: n-the number of studies

Primary sequence survey: first patients conducted pH-metry and then EGDS with biopsy material for testing in HP and histological studies of the stomach mucosa. The study was conducted in the morning on an empty stomach, in 12-14 hours after the last meal. The data obtained were processed statistically using Student t-test with the computation of averages (m) and perhaps the likelihood of deviations (m). The changes were considered to be statistically significant at $p < 0.05$. Statistical calculations were performed using Excel tables for Microsoft Office.

RESULTS AND DISCUSSION

Data obtained during the conduct of pH-testing are shown in table I.

When carrying out the EGDS active ulcerous process in the duodenal ulcer was diagnosed at 11 (4.5%), and 23 (9.4%) patients had manifestations of carried in the past ulcers duodenal bulb as scar deformity of varying degrees of severity. When analyzing data of histological investigations of all patients in 100% of cases, have confirmed the existence of chronic non-atrophic gastritis in both active and inactive stages of varying degrees of severity.

When testing on HP *Helicobacter* infection was detected in 100% of cases when the degree for semination from (+) to (+++). Data on the extent of the gastric mucosa for semination of HP infection on topographical zones of the stomach patients with chronic non-atrophic gastritis are reflected in table II.

During comparative analysis of data on the medium level semination with HP infection of gastric mucosa on topographical zones reliable differences weren't found ($p > 0.05$).

When analyzing data by frequency of held appendectomies it was found that 56 (22.9%) patients had such an operation. Data on the age of patients during appendectomy and age, when we started the first pathological manifestations of the gastro-intestinal tract after operation, are presented in table III.

Age of patients, who had an appendectomy ranged from 4 to 40 years and averaged 18.34 ± 1.05 years, and the first pathological manifestations of the gastro-intestinal tract appeared in an average of 28.27 ± 1.75 year, i.e. in 10 years. As for the age qualification pupil were the earliest pathological manifestations appeared in a group of patients from 11 to 15 years and amounted to about 6 years after the operation, and 6 (46.2%) patients, manifestations appeared in 2-6 months after surgery; the most recent is in group from 16 to

20 years and amounted to about 14 years ($p < 0.05$). The first manifestations in the form of proved chronic gastritis was 42 (75%), duodenal ulcer-7 (12.5%), acute pancreatitis-4 (7.2%) chronic colitis-2 (3.6%), cholelithiasis-1 (1.7%) patient.

These results are understandable from the point of view of age qualification of having appendectomy. The period from 11 to 15 years is the period of puberty, when hormonal changes are destabilizing the immune system [3, 14, 15]. Operative intervention during this period on the body of immune system strengthen this process that leads to activation of *Helicobacter pylori* infection, because of the two constraints-immunity and acid-peptic factor the first crashes that allows HP infection use more their energy for reproduction. The increase in mass of HP infection activates the inflammatory process in the stomach lining which leads to a primary manifestation of chronic non-atrophic gastritis, which up to this point could be latent [16].

CONCLUSIONS

Surgery on the body of immune system – appendix provokes activation of latent form of chronic non-atrophic gastritis, especially during puberty.

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ORIGINAL ARTICLE

THE FREQUENCY OF VISCERAL AND PHENOTYPIC MARKERS IN PATIENTS WITH THE COMBINATION OF UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE AND GASTROESOPHAGEAL REFLUX DISEASE

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ABSTRACT

The aim: The aim of the research was to study the prevalence of visceral and phenotypic markers of UCTD syndrome in patients with GERD for the purpose of early diagnosis of this comorbidity.

Materials and methods: The study included 120 patients: 75 patients (Group II) – GERD was on the background of UCTD, 45 (Group I) – the patients with GERD. The average age of the patients was 42.05 ± 6.5 years. Evaluations of UCTD's were performed accordingly to the criteria recommended by M. Moska et al., A. Doria et al., T. I. Kadurina, L. M. Abbakumova in the modification of T. Milkovskaya-Dimitrova, and the degree of their expression on the scale of T. Y. Smolnova.

Results: Among the examined patients, the specific criteria for the certain connective tissue diseases were detected in the patients with UCTD from 2.7 and 20.0% more often. Bone, joint and skin phenotypic signs of dysplasia were observed in patients with GERD associated with UCTD by 4–4.5 times more often. Various abnormalities of internal organs development were detected in the majority of patients of Group II, namely in 88.0%, and only in 6.6% of the patients of Group I. According to the data on daily pH monitoring, esophagus AET constituted 4.6% of the total monitoring period in Group I and 5.48% in the patients of Group II. The number of refluxes with $\text{pH} < 4$ recorded in the patients of Group I constituted 57 ± 8 episodes, and 79 ± 6 episodes in the patients of Group II.

Conclusions: The obtained data indicated that the number of pathological GER was significantly higher in the setting of comorbidity. Our research also showed that the chances of diagnosing Reynaud's Syndrome, arthralgia, unmotivated body weight loss, dysphagia, skin rash, oral ulcers, proximal muscle weakness in the patients with GERD associated with UCTD are higher in comparison with the patients with GERD without comorbidity ($p < 0.05$). This should necessarily be taken into account in the early diagnosis and when assigning a complex therapy in case of this pathology.

KEY WORDS: gastroesophageal reflux disease, undifferentiated connective tissue dysplasia, visceral and phenotypic markers

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INTRODUCTION

Modern medicine is special by the reason that diseases lose their mono- nosological character and more often manifest in comorbide course. In recent decades, the incidence of undifferentiated connective tissue disease (UCTD) has increased in Ukraine, with a frequency of diagnosis from 9% to 85% of cases. UCTD refers to unclassified systemic autoimmune diseases. Such diagnosis is made if the patient has had signs and symptoms indicative of connective tissue lesions for three years or more but which do not correspond in their set to the criteria for any of differentiated connective tissue diseases in the presence of low titer of antinuclear antibodies in blood serum [1-6]. These data are also confirmed by the Project Regulation ReCONNET of the European Reference Networks (ERN) according to which the issue of referring patients to UCTD group is possible if its manifestations are not specific to such connective tissue diseases as rheumatoid arthritis, scleroderma, widespread vasculitis, myositis, systemic lupus erythematosus, Sjogren's syndrome [1,7,8].

According to the scientific data, UCTD can cause dysplastic changes in the connective tissue of various organs and systems and, accordingly, be a premorbid background for the development of many pathological conditions and chronic diseases. The digestive system is the second by the highest level of collagenization after the cardiovascular system, changes in it are particularly often observed in case of UCTD. It causes changes in the length and size of the digestive system and the esophagus first of all, which is likely to be due to its mesenchymal origin [7]. According to the scientific studies, gastrointestinal tract lesions were observed in 80% of patients in the German national cohort, and changes in the esophagus were noted most often [9]. In particular, gastroesophageal reflux disease (GERD) was diagnosed in 29.0% of patients with UCTD, while it was observed only in 2.0% of the examined patients in case of UCTD absence [10]. This syndrome changes the clinical findings of the underlying disease, promotes its prolonged and atypical course, low effectiveness of the therapy, complications and disability in the young age [11].

THE AIM

The aim of the research was to study the prevalence of visceral and phenotypic markers of UCTD syndrome in patients with GERD for the purpose of early diagnosis of this comorbidity.

MATERIALS AND METHODS

The research was conducted at two settings: the Therapeutic Department of the University Clinic of Ivano-Frankivsk National Medical University (IFNMU) and the Therapeutic Department №2 of Ivano-Frankivsk Central City Clinical Hospital. During 2016-2019, 120 patients were examined in both centers according to a single algorithm (65 men and 55 women with an average age of 42.05 ± 6.5 who underwent inpatient treatment of GERD. GERD developed on the background of UCTD syndrome in 75 patients (Group II) and no signs of connective tissue dysplasia in case of GERD were noted in 45 patients (Group I). 25 men and 20 women were examined in Group I and 40 men and 35 women were examined in Group II.

Targeted search for visceral and phenotypic signs of UCTD syndrome was performed in each patient in addition to traditional clinical and laboratory-instrumental research methods. They were evaluated according to the criteria recommended by Moska et al [1999, 2006], A. Doria et al [2005], expert tables with a score assessment of the relevance of a number of clinical, laboratory and instrumental CTD indicators recommended by T.I. Kaduryina (2009), L.N. Abakumova in the modification by T. Milkovska-Dymytrova, the degree of their severity according to the scale by T.Yu. Smolnova [2009]. The presence of joint hypermobility signs was also determined according to Carter & Wilkinson criteria (1964) modified by Beighton (1983) [12,13]. The maximum value of the indicator was 9 points in case of bilateral localization. The state of hypermobility was determined when scoring from 4 to 9 points. Walker-Murdoch sign (put together thumb and little finger totally encircle the wrist) and thumb sign were additionally detected.

The research was approved by the Bioethics Committee of Ivano-Frankivsk National Medical University and conducted according to the principles of the Helsinki Declaration. All patients signed a voluntary informed consent before the study.

GERD was diagnosed according to the criteria of the unified clinical protocol (the Order of the Ministry of Health of Ukraine from October 31, 2013 № 943) taking into account complaints, the data of endoscopic examination, results of daily intragastric pH-metry. Criteria for inclusion in the study were the presence of typical GERD clinical manifestations such as heartburn, regurgitation, pain in the epigastric region and behind the sternum, dysphagia, dry cough, esophagitis of different degrees according to the results of esophagogastroduodenoscopy (EGD). Los Angeles (LA) classification (1998) was used for the endoscopic assessment of the degree of esophagus lesion. The exclusion criteria included peptic ulcer, surgical

intervention on the gastrointestinal tract organs; malignant lesions of any localization, prolonged use of nonsteroidal anti-inflammatory drugs, diabetes mellitus, pregnancy, lactation, severe somatic diseases.

Daily pH monitoring (according to Professor V.N. Chernobrovyi's method) was conducted in order to determine pathological gastroesophageal reflux (GER). Introduced transnasally pH- microprobe was used in the course of examination. The electrode was placed 5 cm above the proximal edge of the lower esophageal sphincter. The patients were recommended to stop using coffee, fruit juices and antacids, and they were suggested to keep a diary of events, including a clear indication of the hour of reflux symptoms. pH-metry data were registered by the portable apparatus Acidogastrograph "AG-1pH-M" (Ukraine).

The statistical analysis of the results was performed using STATISTICA 7.0 software packages and the package of statistical functions of Microsoft Excel. Arithmetic mean (M) and standard error ($\pm m$) were used to describe the quantitative characteristics. The reliability of the obtained indices was confirmed by calculating the errors for relative values, and the probability of the difference in the data in the comparable groups was proved on the basis of the calculation of t-coefficient (Student t-test) and determination of precise prognosis according to the accuracy table. The comparison of qualitative features was performed by chi-square method calculation using the analysis of chi-square distribution table. The odds ratio (OR), the standard error of odds ratio (S) were calculated, the statistical significance of this connection was evaluated by calculating 95% of its confidence interval (CI): its lower and upper boundaries. The indicator of 0.05 was considered to be the critical level of significance when checking the statistical hypotheses.

RESULTS AND DISCUSSION

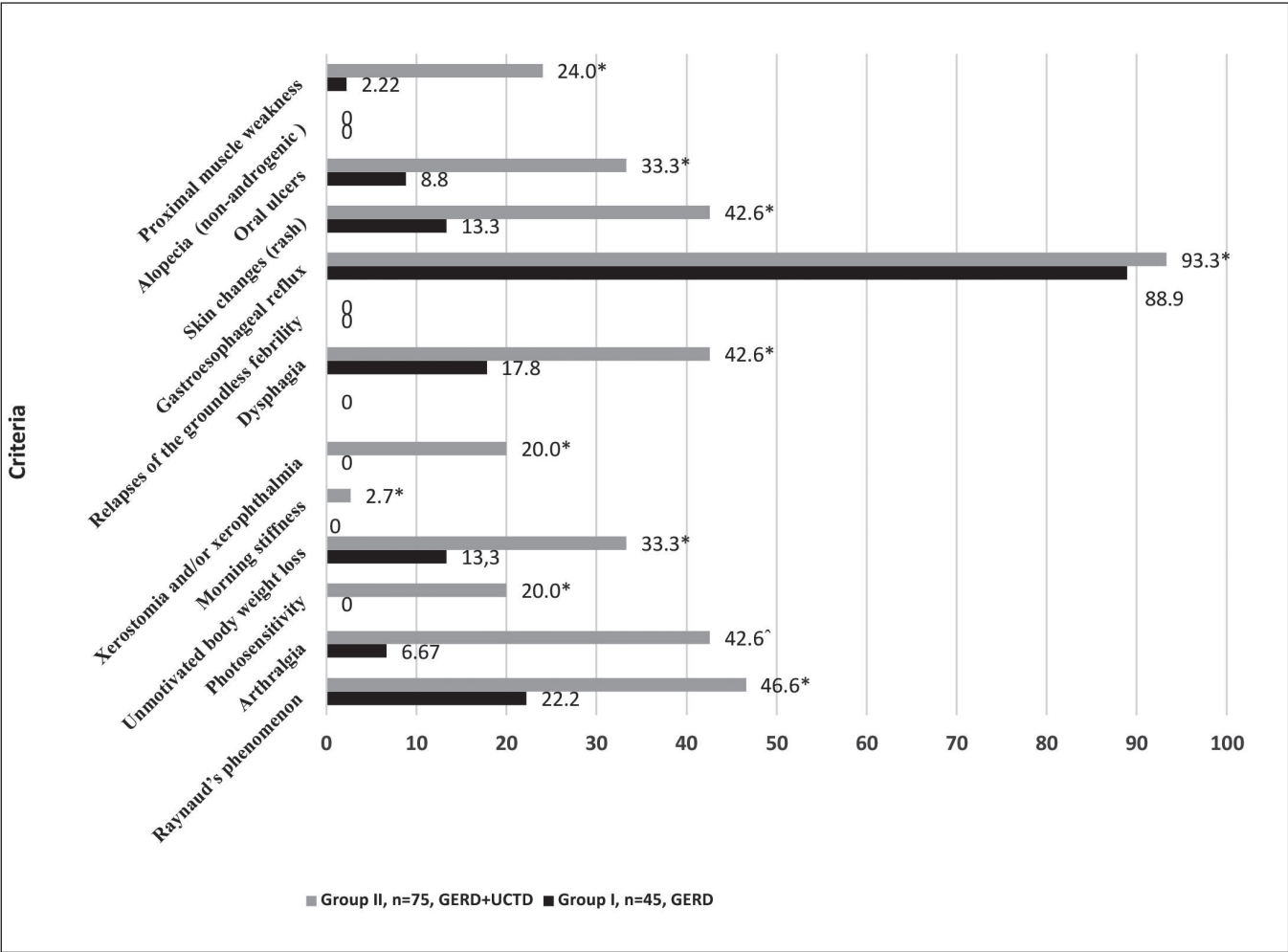
The data on visceral and phenotypic signs of UCTD in the examined patients are presented in Table I and Table II.

According to the data presented in Table I., the abnormalities of the internal organs development were detected in 66 (88.0%) patients of Group II forming the majority, while they were observed only in 3 (6.6%) patients of Group I.

In particular, hiatal hernia was diagnosed in 20.0% of patients with comorbid pathology. Cardia insufficiency was recorded 2.75 times more often in the patients with GERD developed on the background of UCTD. The examined patients with UCTD were also diagnosed with ptosis of the transverse colon by 3.7 times more frequently, dolichosigmoid by 2.6 times more often, colon diverticulum by 7 times. Ileocecal valve insufficiency was determined in 9.3% of cases in the patients with comorbid pathology. UCTD often manifested in abnormalities and functional disorders of the biliary system which may be the basis for duodenogastric reflux development. Nephroptosis and ectopic kidney (15.5% vs. 46.6%), pelvic ptosis (22.2% vs. 80.0%) occurred also more frequently in the patients with this comorbidity. Mitral valve prolapse was diagnosed in 49

Table I. Visceral signs of UCTD in the patients examined for gastroesophageal reflux disease and its combination with undifferentiated connective tissue dysplasia

Visceral signs of UCTD	Group I GERD		Group II GERD + UCTD		χ^2	px^2
	abs	%	abs	%		
Cardia insufficiency	8	17.7	22	29.3	2.003	0.157
Diaphragmatocele	-	-	15	20.0	10.286	0.002
Gastroptosis	7	15.5	13	17.3	0.064	0.801
Ptois of the transverse colon	3	4.5	11	14.7	1.747	0.187
Biliary dyskinesia	8	17.7	25	33.3	3.413	0.065
Gallbladder abnormalities	5	11.1	65	86.6	66.057	<0.001
Dolichosigmoid	8	17.7	21	28.0	1.604	0.206
Colon diverticulum	2	4.4	14	18.7	4.923	<0.05
Ileocecal insufficiency	-	-	7	9.3	4.460	<0.05
Nephroptosis and hepatic dystonia	7	15.5	35	46.6	11.966	<0.001
Pelvic ptosis	10	22.2	60	80.0	38.629	<0.001
Supplemental chord of the left ventricle	4	8.8	35	46.6	18.297	<0.001
Mitral valve prolapse	7	15.5	49	65.3	28.000	<0.001
Abnormal kidney development	2	4.4	24	32	12.583	<0.001



Note: The data are reliable between the study groups: * – ($p<0.05$); ^ – ($p<0.001$).
Fig.1. The frequency of UCTD criteria detection in the patients with GERD and its combination with this pathology according to M. Moska et al [1999, 2006, 2014] and A. Doria et al. [2005].

Table II. Phenotypic signs of UCTD in the examined patients according to the criteria of T.I. Kadurina, L. N. Abakumova in the modification of T. Milkovska-Dymytrova [2008]

Clinical groups	Group I GERD (n=45)		Group II, GERD + UCTD (n=75)		χ^2 df=1	p χ^2
	abs.	%	abs.	%		
Phenotypic signs of UCTD						
	Bone:					
Asthenic bodybuild	9	20.0	66	88.0	55.488	<0.001
Arachnodactyly	7	15.5	66	88.0	61.950	<0.001
Vertebral deformity: scoliosis/ kyphosis	6	13.3	60	80.0	50.505	<0.001
Chest distortion	5	11.1	45	60.0	27.657	<0.001
Cranial deformation	3	6.66	15	20.0	3.922	0.048
Sandal gap	-	-	5	6.66	3.130	0.077
Flat feet	8	17.7	32	42.66	7.840	0.006
High roof of the mouth or Gothic palate	5	11.1	15	20.0	1.600	0.206
Abnormality of occlusion	4	8.8	9	12.0	0.282	0.596
Walker-Murdoch sign	12	26.6	60	80.0	33.333	<0,001
The predominance of finger IV length over finger II	9	20.0	35	46.6	8.612	0.004
Dentition out of arch, hyperdontia	4	8.8	26	34.6	9.967	0.002
Anterior diastema	3	6.6	9	12.0	0.889	0.346
Defective mandibular bone	1	2.22	12	16.0	5.527	0.019
	Skin:					
Skin hyperelastosis (from 3 cm)	5	11.1	32	42.6	13.132	<0.001
Delicate, velvety, tender skin	4	8.8	26	34.6	9.967	0.002
Nevus pigmentosis	6	13.3	68	90.6	71.154	<0.001
Percutaneous pseudoneoplasms and spheric masses	1	2.22	5	6.66	1.170	0.280
Atrophic striae (not related to pregnancy or obesity)	5	11.1	12	16.0	0.553	0.458
Formation of keloid scars	4	8.8	24	32.0	8.398	0.004
Herniae and organ prolapse	3	6.66	13	17.3	2.769	0.097
Muscular hypotonia and/or hypertrophy	5	11.1	19	25.3	3.556	0.060
Epicanthal fold	-	-	5	6.66	3.130	0.077
Hanging uvula	-	-	7	9.33	4.460	0.035
Ability to roll the tongue into a tube	6	13.3	25	33.3	5.872	0.016
Angiectasia, hematoma in case of minor blows	3	6.66	12	16.0	2.240	0.135
Nasal septum deviation	7	15.5	65	86.6	71.154	<0.001
Auricle abnormalities	3	6.66	25	33.3	11.180	<0.001
	Joints:					
Joint hypermobility	2	4.44	45	60.0	36.433	<0.001
Hip dysplasia	-	-	3	4.0	1.846	0.175
Ligament rupture in past medical history	1	2.22	9	12	3.520	0.061
Common joint dislocation	-	-	2	2.66	1.220	0.270

(65.3%) patients in Group II and only in 7 (15.5%) patients in Group I, and supplemental chord of the left ventricle was detected in 35 patients (46.6%) vs. 4 patients (8.8%). Abnormal kidney development was also more common in the patients with a combination of GERD and UCTD, namely 24 (32%) vs. 2 (4.44%). According to the results of fibrogastroduodenoscopy, the reflux, namely esophagitis,

was significantly more frequent in the patients with such combined pathology.

Such phenotypic signs of UCTD as asthenic bodybuild, arachnodactyly, vertebral deformity and chest distortion, sandal gap, joint hypermobility, skin hyperelastosis, keloid scars on the skin, hanging uvula, high roof of the mouth being the main signs of UCTD were observed from 6.6 to

Table III. The odds ratio for UCTD criteria present in the patients with GERD and its combination with this pathology according to M. Moska et al [1999, 2006] and A. Doria et al [2005]

Nº	Criteria	Group I, n=45 GERD	Group II, n=75 GERD +UCTD	$p\chi^2$ (df=1)	OR	S	95 % CI OR
1	Raynaud's phenomenon	10 (22.2%)	35 (46.6%)	0.008	3.063	0.427	1.327- 7.069
2	Arthralgia	3 (6.67%)	32 (42.6%)	<0.001	10.419	0.642	2.963-36.639
3	Photosensitivity	–	15 (20.0%)	0.002	–	–	–
4	Unmotivated body weight loss	6 (13.3%)	25 (33.3%)	0.016	14.375	0.521	5.180–39.890
5	Morning stiffness	–	2 (2.7%)	0.270	–	–	–
6	Xerostomia and/or xerophthalmia	–	15 (20.0%)	0.002	–	–	–
7	Dysphagia	8 (17.8%)	26 (42.6%)	0.047	11.461	0.481	4.468–29.399
8	Relapses of the groundless febrility	–	–	–	–	–	–
9	Gastroesophageal reflux	40 (88.9%)	70 (93.3%)	0.394	1.750	0.663	0.477–6.415
10	Skin changes (rash)	6 (13.3%)	32 (42.6%)	<0.01	28.308	0.538	9.864–81.240
11	Oral ulcers	4 (8.8%)	25 (33.3%)	0.003	22.188	0.595	6.912–71.224
12	Alopecia (non-androgenic)	–	–	–	–	–	–
13	Proximal muscle weakness	1 (2.22%)	18 (24.0%)	0.002	49.333	1.052	6.279–387.6

26.6% of cases in the patients of Group I and from 20% to 92.0% in the patients of Group II. At the same time, bone symptom complex was noted in such patients, 5 out of 14 signs of which were more significantly prevalent in the patients with comorbid pathology and included the asthenic bodybuild ($\chi^2 = 55.488$; $p\chi^2 < 0.001$); arachnodactyly ($\chi^2 = 61.950$; $p\chi^2 < 0.001$); vertebral deformity ($\chi^2 = 50.505$; $p\chi^2 < 0.001$), chest distortion ($\chi^2 = 27.657$; $p\chi^2 < 0.001$). Skin symptom complex ranked second, and 4 out of 14 its signs such as skin hyperelastosis ($\chi^2 = 13.132$; $p\chi^2 < 0.001$), nevus pigmentosus ($\chi^2 = 71.154$; $p\chi^2 < 0.001$), nasal septum deviation ($\chi^2 = 71.154$; $p\chi^2 < 0.001$), auricle abnormalities ($\chi^2 = 11.180$; $p\chi^2 < 0.001$) were the most characteristic for the examined patients with this comorbidity. Joint hypermobility syndrome ($\chi^2 = 36.433$; $p\chi^2 < 0.001$) was significantly more often observed in the patients with GERD associated with UCTD.

The frequency of M. Moska and co-authors' criteria is provided in Fig. 1.

According to the figure, Raynaud's phenomenon was more often detected in the patients of Group II in comparison with Group I ($p < 0.05$). Arthralgia was diagnosed with even higher incidence in the patients of Group II, namely by 35.93% compared to Group I ($p < 0.001$), and dysphagia was detected by 24.8% more often, respectively ($p < 0.05$). Periodic skin rash was more frequent by 29.3% and oral mucosa ulcers were detected by 24.5% more often in the patients of Group II ($p < 0.05$). As a matter of interest, proximal muscle weakness was observed in 2.22% of patients with GERD which was less than 21.78% ($p < 0.05$) in comparison with the patients who suffered from this disease associated

with connective tissue dysplasia. Groundless febrility and non-androgenic alopecia were not detected in any of the experimental groups. Symptoms such as morning stiffness were present only in 2.7% of patients, xerostomia and/or xerophthalmia was observed in 20.0% of cases, photosensitivity was noted in 20.0% of patients with comorbidity. The above mentioned criteria were absent in the patients of Group I.

We calculated the odds ratio with 95% confidence interval for the purpose of numerical expression of how the absence or presence of concomitant pathology was related to the presence or absence of a certain criterion in each of the experimental groups. The obtained data are presented in Table III.

Thus, according to the study, the chances of diagnosing the Raynaud's phenomenon in the patients with GERD associated with UCTD were higher by more than three times ($OR = 3.063$) compared to the patients with GERD without comorbidity. This dependence was statistically significant, since 95% CI was within the range of 1.327–7.069 ($p < 0.05$). The high probability of detecting such criteria for comorbidity as arthralgia ($OR = 10.419$; 95% CI = 2.963–36.639), unmotivated body weight loss ($OR = 14.375$; 95% CI = 5.180–39.890), dysphagia ($OR = 11.461$, 95% CI = 4.468–29.399), skin rash ($OR = 28.308$; 95% CI = 9.864–81.240), oral ulcers ($OR = 22.188$; 95% CI = 6.912–71.224), proximal muscle weakness ($OR = 49.333$; 95% CI = 6.279–387.6) was statistically significant ($p < 0.05$). As a matter of interest, the reason for the fact that the odds ratio of pathological GERD incidence in the patients with and without CTD manifestations was not significantly different ($OR = 1.750$;

95% CI=0.477-6.415) was due to the fact that this criterion was one of the main criteria of the patients' inclusion in our study.

The analysis of transnasal daily monitoring of pH in the lower third of the esophagus in the patients with GERD and its association with UCTD indicated that the general index of pH<4 with the duration above normal was observed in 60% (45) of the patients in Group II and in 26.6% (12) of the patients in Group I. The number of refluxes with pH<4 recorded in the patients of Group I was 57±8 episodes with a total duration of 67±3 minutes. Acid exposure time (AET) in the patients of Group I was 4.6% of the total monitoring period, which was slightly above normal. According to the Lyon Consensus, this parameter is considered to be significantly normal at a value of less than 4% and significantly pathological at a value of more than 6%, and the values within this interval are considered inconclusive and require additional examinations. The number of acid refluxes constituted 79 ± 6 episodes with a total duration of 87±8 minutes, AET = 5.48%, in the patients of Group II. Time share of pH<4 amounted to 3.5 hours in the patients of Group II and 2.9 hours in the patients of Group I ($p<0.05$). The mean De Meester score constituted 23.01±2.24 in the examined patients with GERD and 31.08±2.4 in the patients with GERD associated with UCTD ($p<0.05$). An increase in the percentage of time with pH<4 was also determined in the lower third of the esophagus in the upright position and in lying position in 12.3% and 7.4% of the patients with combined pathology respectively, and in 10.2% and 5.8% of patients with GERD without concomitant disease, respectively. According to the results of the correlation analysis, a direct correlation between the number of refluxes with pH<4 with duration over 5 minutes and the degree of UCTD severity ($r = 0.78$) was established, as well as between the mean indices of the De Meester Score and the degree of UCTD severity ($r = 0.68$). We also monitored the correlation between the indicator of abnormal esophagus acidification (acid exposure time-AET) and the degree of UCTD severity ($r = 0.59$).

We used T. Yu. Smolnova scale in order to assess the aggregate and severity of CTD clinical manifestations. According to the scale, the patient was diagnosed with CTD of mild severity in case of total points up to 9, with CTD of moderate severity in case of total points from 10 to 16, and with severe CTD in case of total points from 17. According to the presented data, the signs of CTD of mild severity were predominant in the patients of Group I, and CTD of moderate severity prevailed in Group II. UCTD of moderate severity was noted in 69.0% of the patients with combined pathology and in only 44.0% of the patients with GERD. Signs of severe UCTD were diagnosed in 4% of patients in Group II and were absent in the patients of Group I.

Thus, UCTD is characterized by the signs reflecting the systemic autoimmune process which does not correspond to any accepted diagnostic criteria for diseases such as rheumatoid arthritis, Sjogren's syndrome, systemic

scleroderma, polymyositis and dermatomyositis, systemic lupus erythematosus, and mixed connective tissue diseases. Among the examined patients, the specific criteria for the above mentioned diseases were detected in the patients with UCTD from 2.7 and 20.0% more often. Bone, joint and skin phenotypic signs of dysplasia were observed in patients with GERD associated with UCTD by 4-4.5 times more often. Summarizing the obtained data, it is necessary to consider the fact that there are many people with insignificant, barely noticeable phenotypic manifestations of dysplasia, but with many visceral signs of connective tissue weakness. This is confirmed by the fact that various abnormalities of internal organs development were detected in the majority of patients of Group II, namely in 88.0%, and only in 6.6% of the patients of Group I. The obtained data on the visceral markers of undifferentiated connective tissue dysplasia were consistent with the results of studies by other authors [15,16].

According to the data on daily pH monitoring, esophagus AET constituted 4.6% of the total monitoring period in Group I and 5.48% in the patients of Group II being 1.19 times higher which is above normal according to the Lyon Consensus 2017 (LC-2017). In accordance with this Consensus, this parameter is considered to be significantly normal at a value of less than 4%, and significantly pathological at a value above 6%. If this parameter is within this interval, i.e. it falls within the limit of "unconvincing values", the number of refluxes per day are then additionally assessed [14]. The number of refluxes with pH<4 recorded in the patients of Group I constituted 57±8 episodes, and 79±6 episodes in the patients of Group II. According to the Lyon Consensus, more than 80 refluxes are considered significantly increased, and less than 40 refluxes are considered a physiological norm.

CONCLUSIONS

The obtained data indicated that the number of pathological GER was significantly higher in the setting of comorbidity. Our research also showed that the chances of diagnosing Reynaud's Syndrome, arthralgia, unmotivated body weight loss, dysphagia, skin rash, oral ulcers, proximal muscle weakness in the patients with GERD associated with UCTD are higher in comparison with the patients with GERD without comorbidity ($p<0.05$). This should necessarily be taken into account in the early diagnosis and when assigning a complex therapy in case of this pathology.

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ORIGINAL ARTICLE

APPLICATION OF NEURAL NETWORK TECHNOLOGIES IN THE DENTAL CARIES FORECAST

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The aim: of the work was to develop and apply in the clinical trial a software product for the dental caries prediction based on neural network programming.

Materials and methods: Dental examination of 73 persons aged 6-7, 12-15 and 35-44 years was carried out. The data obtained during the survey were used as input for the construction and training of the neural network. The output index was determined by the increase in the intensity of caries, taking into account the number of cavities. To build a neural network, a high-level Python programming language with the NumPy extension was used.

Results: The intensity of carious dental lesions was the highest in 35-44 years old patients – 6.69 ± 0.38 , in 6-7 years old children and 12-15 years old children it was 3.85 ± 0.27 and 2.15 ± 0.24 , respectively ($p < 0.05$). After constructing and training the neural network, 61 true and 12 false predictions were obtained based on these indices, the accuracy of predicting the occurrence of caries was 83.56%. Based on these results, a graphical user interface for the “CariesPro” software application was created.

Conclusions: The resulting neural network and the software product based on it permit to predict the development of dental caries in persons of all ages with a probability of 83.56%.

KEY WORDS: caries, prognosis, neural network, software

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INTRODUCTION

Innovative and promising research areas include the use of intelligent systems, in particular, those based on neural networks [1,2,3]. In health care, such systems are used in human genome research, drug development, diagnosis and prognosis of certain diseases, such as cancer, cardiovascular diseases, etc. [2,3,4,5]. In the processing of medical data, information systems based on neural networks have several advantages over traditional methods of mathematical statistics. Neural networks are capable of simultaneous processing a large array of different parameters, which is extremely important in the diagnosis and prognosis of diseases, particularly if the patient has several diseases. In such systems, after their creation, there is a possibility for “extra training” of the initial model, for example, with the emergence of new or more accurate data of additional studies or “extra training” of the model on the initial data in changing external conditions, for example, in the case of new drugs prescribing during treatment [1,4,5].

The work of neural networks is based on algorithms that are, in their turn, based on the principles and patterns of signal transmission through neurons and synapses of the human nervous system. Neural networks are capable of taking decisions and returning results based on the hidden patterns found in the large data array. The peculiarity of neural networks is that they are not programmed and do not use any rules to draw a conclusion, but they are trained to do it by examples [1,6]. Thus, neural network

technologies can be applied to solve difficult and monumental challenging tasks with numerous parameters and characteristics in various fields of healthcare.

State of the present-day health care is drawing attention to preventive care. Against this background, it is quite promising and, certainly, important to predict the development and course of diseases, in particular dental ones, among which, undoubtedly, the leading place is occupied by dental caries and its complications [7,8]. To date, the prevalence and intensity of this disease in pediatric and adult populations in different regions of Ukraine remain at a fairly significant level [8,9,10]. Integrated caries prevention measures applied to different segments of the population, including organized contingents, taking into account regional peculiarities, do not always show high efficiency due to, first of all, the lack of an individualized approach. Caries prevention, based on the principles of selectivity, i.e. sampling, and timeliness, based on determining the individual disposition toward caries, creates the preconditions for improving its efficacy, for more rational use of the dentist's working time as well as therapeutic and preventative means. In this sense, the predictive field of cariesology, i.e. prediction of this disease course and the possible results of preventive measures for a particular patient, seems quite appropriate and necessary.

There are various approaches to the prediction of dental caries in children and adults, well-known are, for example, mathematical models, as well as numerous predictive

methods based on the determining oral fluid properties, the state of the dental hard tissues, hygienic parameters of the oral cavity, etc. [11]. Clinical and laboratory studies of the tooth enamel acid resistance and its restitution after acid exposure are considered to be the most informative and prognostically valuable. These include determination of structural and functional acid resistance of tooth enamel by the enamel resistance test, clinical assessment of enamel remineralization rate, CRT-test (color, reaction, time), but they have recently been used infrequently enough [11,12].

Unfortunately, most of the proposed prediction methods are technically sophisticated, sometimes multi-step and time-consuming, and quite frequently their implementation is not possible in daily clinical dental practice. Therefore, it is important to develop fundamentally new approaches to the prediction of dental caries based on software products, especially since computer technologies are being actively implemented into the medical-diagnostic process, particularly in private-owned dental institutions.

THE AIM

The aim of the work was to develop and apply a software product to predict dental caries on the basis of neural network programming.

MATERIALS AND METHODS

Dental examination of 73 persons was carried out, which was divided into three age groups. The first group included 26 children aged 6-7 years, the second group included 34 12-15 years old children, the third group comprised 13 persons aged 35-44 years. During the examination, the caries intensity in temporary (if present) and permanent teeth was determined according to the index of cfc (caries, filling, cavity) + cfcex (extracted teeth) or CFcEX, taking into account the number of tooth decay cavities, oral hygiene condition according to the simplified hygienic Green-Vermillion index (OHI-S), structurally-functional acid resistance of tooth enamel (SFARE) according to the enamel resistance test (TER) and the functional component of the structural and functional acid resistance of enamel (Δ TER) [13,14]. After 1 year, a repeated clinical examination was performed, each patient was re-assessed by cfc + CFcEX or CFcEX index and the increase in the intensity of carious lesions was calculated according to the Δ cfc + CFcEX or Δ CFcEX index. The results of the study were recorded in the medical card of the dental patient (form No. 043 / o). We used a standard package of statistical software for Microsoft Excel to prepare the patient source data tables for and to carry out calculations.

The following indices were used as input (X) data, on the basis of which the caries probability was predicted: age of the patient (X1), dental caries intensity index taking into account the number of cavities (X2), hygienic index OHI-S (X3), structural- functional acid resistance of tooth enamel (X4) and functional component of structural and functional acid resistance of enamel (X5), i.e., five indices

in total. The output, i.e. final, index (Y) was determined by the increase in the intensity of dental caries, taking into account the number of cavities. The obtained database was divided into two samples – the training and the control ones, which included indices of 65 and 8 patients, respectively.

To build a neural network, we used a high-level Python programming language with the NumPy extension. The following software products were used during the work: Pandas, TensorFlow, Keras, Scikit-learn, Matplotlib, Jupyter Notebook [15].

At the first of the successive stages series, the data were processed and prepared. Initially, using the Pandas electronic library for data analysis and processing, the generated database was read from Excel files, after which the downloaded data was divided into input (X) and output (Y). Further, in order to further normalize the data for each of the factors used, maximum values were determined using the standard NumPy function, according to which the normalization was performed. This was necessary to improve the next neural network training process and to reduce the input to a single computing system.

The final step of this stage was to convert the output coordinate to the unitary code (one-hot encoding) using the standard Scikit-learn function. The use of unitary code in the construction of neural networks is required in cases where the number of outputs corresponds to the number of possible output coordinate classes. When a neural network receives a test sample of factors, the maximum value will be only set to one of the outputs.

At the second stage of the neural network construction its future structure and training was drafted. This was done using the sequential method of describing the neural network using Sequential software. During the work, we created a neural network containing five neurons in the input layer (based on the number of available dental indices), one hidden fully connected layer and an output layer with four neurons. The number of synaptic weights and neurons in the hidden layer was determined according to the Kolmogorov – Arnold – Hecht – Nielsen theorem [15]. A ReLU was selected as the activation function for the input layer, and the softmax logistic function – for activation of the output layer. In order to avoid the problem of the neural network “retraining”, which may cause the dependence of the output index on the input data to occur sharply nonlinear, i.e. the result will be significantly and unpredictably changed with slight fluctuations of the input data, the method of “thinning” was applied, better known as Dropout. Its essence is that instead of training a single neural network, there is a simultaneous training of several ones, with further generalization of the results obtained. The RMSprop algorithm was used as the optimization method, for the quality metric of the constructed neural network – Accuracy.

The duration of the neural network training process was 25,000 epochs, with 10% of the available sample data not participating in the process, but used for test purposes only. After training, the neural network and the software

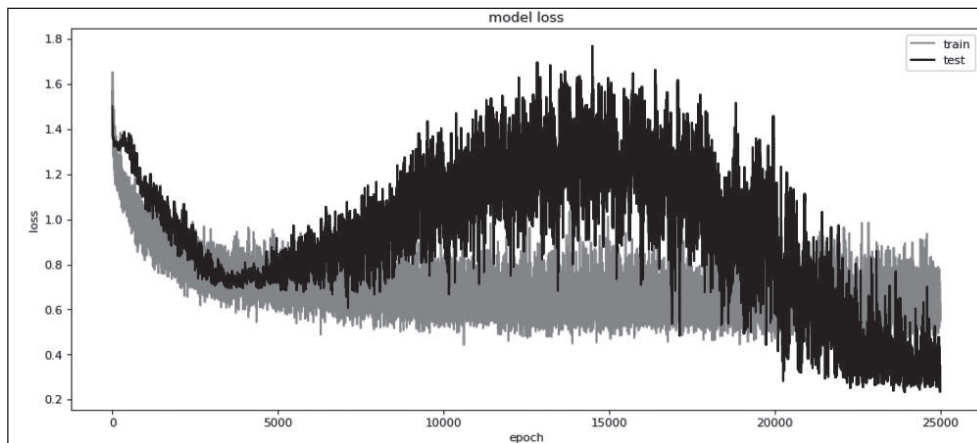


Fig. 1. Diagram of loss-function based on training data (blue - train) and test data (orange - test).

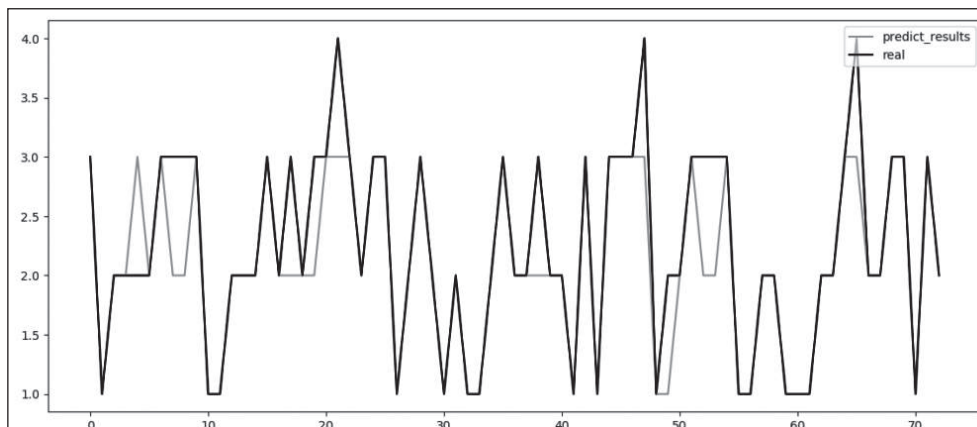


Fig. 2. Diagrams for visualization of the dental caries intensity growth rate prediction based on the developed neural network.

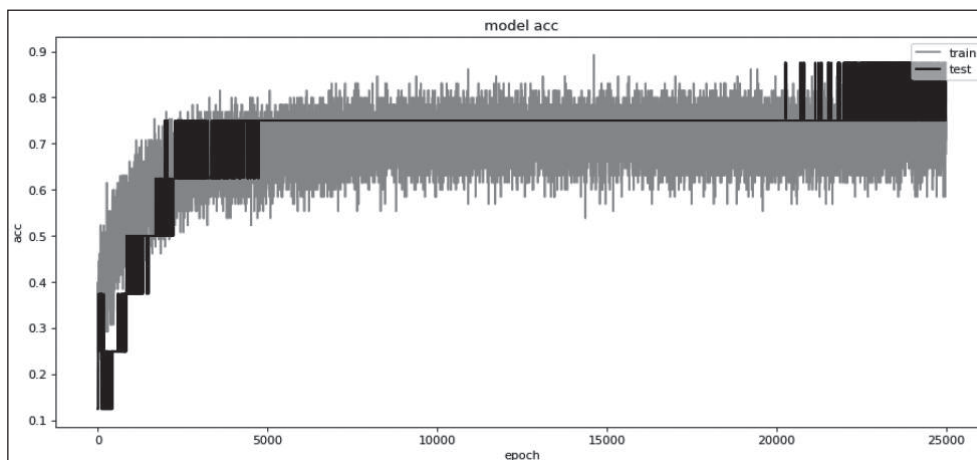


Fig. 3. Visualization of changes in accuracy (acc metrics) in the training process of the resulting neural network.

This is a screenshot of the 'Forecast "CariesPro"' graphical user interface. It features several input fields for patient data, including Last name, First name, Patronymic, Date of birth, Address of residence, Telephone, and Date of visit. There are also fields for analysis data, such as Age, Caries intensity index, Hygiene index DHI-S, TEB, and del TEB. A 'Patient visit to the clinic' section includes a table for recording visits. At the bottom, there are buttons for 'Network calculation', 'Retain', 'Conclusion', 'Monthly visit report', and 'Exit'.

Fig. 4. Graphical interface to the main user form of the "CariesPro" application.

product based on it, called CariesPro, was used to achieve the set purpose, i.e., predicting dental caries. Matplotlib e-library was used to visualize the training outcomes and the results obtained.

All the studies were carried out in accordance with the principles of the Declaration of Helsinki, adopted by the General Assembly of the World Medical Association (1997-2000) and approved by the University Bioethics Commission.

RESULTS

In order to create an input database, a primary examination of persons was carried out, during which it was found that the intensity of tooth decay by $cfc + CFcEX$ index, taking into account the number of cavities in children aged 6-7 years, was 3.85 ± 0.27 , which, according to the WHO standard criteria is considered medium. Statistically significant ($p < 0.05$), this index was lower in 12-15 years old children, in whom it was equal to 2.15 ± 0.24 , which corresponds to the low level according to the WHO recommendations. The intensity of carious lesions in teeth according to the $CFcEX$ index was very high by the WHO criteria in patients aged 35-44 years, it was 6.69 ± 0.38 and statistically significantly ($p < 0.05$) higher than all the others.

Assessment of the oral cavity hygienic condition by the simplified hygienic index OHI-S revealed its unsatisfactory level in individuals of all three age groups, and their mean values were not statistically significantly different ($p > 0.05$), being 1.98 ± 0.05 points, 1.87 ± 0.03 points and 1.86 ± 0.05 points, respectively.

The structural and functional acid resistance of the teeth enamel according to the enamel resistance test was the worst in children aged 6-7 and 12-15 years, in particular, their mean values were 4.96 ± 0.33 points and 5.00 ± 0.34 points ($p > 0.05$), respectively. Somewhat better, but with no statistically significant difference ($p > 0.05$), structural and functional acid resistance of enamel (SFARE) was in patients of the older age group, making 4.15 ± 0.66 points.

After carrying out hemostimulation of the dental pulp, which was performed by rubbing hypertonic sodium chloride solution into the palatal surface of the tested tooth for 10 minutes, the most significant dynamics of structural and functional acid resistance was recorded in 6-7 and 12-15 years old children, who had a functional SFARE component, 1.73 ± 0.15 points and 1.71 ± 0.12 points ($p < 0.05$), respectively [13].

Statistically significantly ($p < 0.05$) less, in particular, almost by 11.5 times, this index was found in persons of 35-44 years – 0.15 ± 0.10 points. Such differences are undoubtedly related to the age patterns regarding the dynamics of the enamel acid resistance and the dominance of its structural component in the elderly patients [14].

After 1 year, the intensity of dental caries in children aged 6-7 years increased by 1.6 times to 6.27 ± 0.35 , in 12-15 years old children this index increased almost by 2 times to 4.23 ± 0.24 , in persons aged 35-44 years – up to 8.92 ± 0.52 , i.e. only by 1.3 times. In all the age groups surveyed,

the indices in the re-survey were statistically significantly ($p < 0.05$) different from the primary ones. The increase in the intensity of dental caries in these individuals by the indices $\Delta cfc + CFcEX$ or $\Delta CFcEX$ was 2.42 ± 0.15 ; 2.09 ± 0.15 and 2.23 ± 0.25 , respectively, that is, indices did not differ statistically ($p > 0.05$).

The results obtained during the two surveys were used to train the neural network. The training outcomes were visualized by constructing a loss function in Matplotlib. This function was used to calculate the error between the existing and the obtained result, i.e. its ultimate purpose is to reduce this error. Thus, the loss function of both in the training data that participated in the training process and in the test data that did not participate in the training process was minimized (fig. 1). This testified to the ability of the neural network to learn. Significant fluctuations in loss functions during the transition from epoch to epoch occurred due to the use of Dropout.

After the process of training the neural network, a whole selection of the reported factors was provided. In response, it has generated a category of numbers for the individual increase in caries intensity (Y), among which index 4 was never met. This is due to the imbalance of the training sample and to the very poor representation of this result in it.

The graphical overlay of the predicted results and the real results diagrams, which were constructed in the same coordinate system, indicated a coincidence of the results (fig. 2).

Visualization of the accuracy of the constructed Accuracy (acc) neural network, which was calculated using Keras, has confirmed the correct course of the neural network training process, and oscillations during the transition from epoch to epoch were due to the use of Dropout (fig. 3).

As a result of calculating work accuracy index of the Accuracy (acc) neural network, 61 true and 12 false predictions were obtained from the entire sample, i.e. the prediction accuracy of the constructed and trained neural network was 83.56%.

Later, using the high-level Delphi programming language, a graphical user interface was created (fig. 4).

In order to work with the presented software, it is necessary to fill in the patient's passport data and enter the five dental indices obtained during the clinical dental examination. The following buttons are present in this window:

“Network calculation” – when this button is pressed, the value of the probable number of carious cavities index of the patient is calculated according to the set values in the analysis data;

“Save” – pressing this button saves patient data and estimated values;

“Monthly visit report” – pressing this button generates a report on the status of teeth and oral hygiene in patients who visited a dentist in the last month;

“Conclusion” – pressing this button generates a conclusion that contains data on the condition of the teeth and oral hygiene during the current and past examinations of the patient, as well as the results of predicting the probable number of caries cavities, based on the obtained dental indices.

DISCUSSION

As of today, there is a large number of computer software to predict dental caries, most of which are based on standardized multifactorial questionnaires [16]. The most famous of them are NusCra (National University of Singapore Caries Risk Assessment), CARIOGRAM, PreViser, ADA (caries risk assessment by the American Dental Association), CAMBRA (Caries Management By Risk Assessment), CAT (America Academy of Pediatric Dentistry's Caries Assessment Tool). Each software takes into account nine to twelve factors, among which, besides clinical, are socio-demographic (age, ethnicity), behavioral factors (diet) and others.

One of the most widely used software in the world, called "CARIOGRAM", is based on the multifactorial analysis of factors that, from the developers' point of view, are leading in the caries development [17]. The software takes into account ten factors, including the salivation rate, the buffer capacity of the oral fluid, the content of lactobacilli and streptococci in it, etc. Identifying some of them requires laboratory testing, additional equipment, and therefore unnecessary financial and time costs. The significance of each factor is conditionally estimated from 0 to 3 points, without relying on any quantitative criteria, which should certainly be considered subjective enough.

The result is a graphical representation of a sectoral diagram including five parts of different colors and only demonstrating the influence of etiological factors. The risk of developing caries, according to the image, is rated as high, medium or low, but the percentages given do not answer the question of the number of possible dental caries lesions in a particular patient over time.

The developed "CariesPro" computer software does not have the above disadvantages. In order to perform full-time work with this software, one needs to identify only five, which is important, exclusively clinical indices that can be established during a single dental examination, so that additional examinations and visits are not required. In addition, the software takes into account the exact figures regarding the condition of the teeth and oral hygiene, which are specific to the particular individual.

The final index in working with the "CariesPro" software is the prediction of a certain number of carious cavities probability after a certain time, which permits to plan and, if necessary, timely carry out the most individualized measures of the selective caries prevention. "CariesPro's" high level of forecasting performance is in line with existing software products, and the said software has the ability to constantly learn and improve.

CONCLUSION

The resulting neural network obtained and the software product based on it permit to predict the development of dental caries in persons of all ages with a probability of 83.56%. Thanks to the developed form, the presented software can be installed in a personal computer, which, in turn, permits its efficient use for predicting dental caries in daily clinical practice.

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ORIGINAL ARTICLE

ASSESSMENT OF ESTROGEN RECEPTOR GENE POLYMORPHISM (T-397C VARIANT) IN PATIENTS WITH PREMENSTRUAL SYNDROME

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ABSTRACT

The aim: of the study is to determine the frequency of polymorphism of estrogen receptor gene ESR1 (T-397C variant) in patients with premenstrual syndrome.

Materials and methods: 50 women with diagnosis of premenstrual syndrome (the basic group) and 25 persons without it (the control group) were examined. Polymerase chain reaction was used to study T-397C polymorphism of estrogen receptor gene ESR1.

Results: There was no significant difference in allele and genotype rates of ESR1 gene between persons with premenstrual syndrome and controls. TT genotype was determined in 24.0 % women in the control group and 24 % of patients in basic group (OR=1.00, 95 % CI=0.32-3.08, p=1.00), TC genotype – in 52.0 % and 46.0 % of individuals respectively (OR=0.79, 95 % CI=0.30-2.06, p=0.62), CC genotype – 24.0 % and 30.0 % of women respectively (OR=1.36, 95 % CI=0.45-4.07, p=0.59). Also, the frequency of T allele and C allele was similar in individuals with pathology and healthy women. There was no significant difference in allele and genotype rates of T-397C variant of ESR1 gene between patients with mild and severe forms of premenstrual syndrome and controls.

Conclusions: There is no association of T-397C polymorphic variant of estrogen receptor gene ESR1 with the development of premenstrual syndrome.

KEY WORDS: premenstrual syndrome, estrogen receptor, polymorphism

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INTRODUCTION

Premenstrual syndrome (PMS) is a common neuroendocrine syndrome in gynecology. 95 % of women of reproductive age suffer from this pathology, these data differ and depend on the population, ethnic group, age, used diagnostic criteria [1]. The complex of numerous physical and psychological symptoms which occur in the second phase of the menstrual cycle and lead to daily functional impairment composes PMS. Stress load, poor physical activity, smoking, alcohol contribute to the forming of PMS [2]. The decline of quality of life – decreased daily and professional activities are usually observed in women with this disease [3]. Thus, PMS can be considered as the biopsychological pathology [4].

Different pathogenetic mechanisms are described to explain all clinical manifestations of PMS. The changes of estrogens and progesterone (its metabolites) levels during the menstrual cycle often lead to clinical signs of the syndrome. Besides this, these hormones considered to be as neuromediators and significantly affect mood changes [5] that can cause psychological manifestations of the pathology. In addition, the role of serotonergic regulation, renin-angiotensin system are important in the development of PMS. Genetic aspects of PMS development are less studied. Certainly, family history has the important meaning. The significance of gene polymorphism is not

established well. Nowadays the role of serotonin transporter gene polymorphism in the genesis of premenstrual dysphoric disorder was demonstrated [6].

THE AIM

The aim of the study is to determine the frequency of T-397C polymorphism of estrogen receptor gene ESR1 in patients with PMS.

MATERIALS AND METHODS

The study included 50 women with the diagnosis of PMS who formed the basic group, 25 of them had mild form of PMS and 25 – severe one. 25 women without this pathology were controls. The research was carried out in Ivano-Frankivsk City Clinical Perinatal Centre (Ukraine). Diagnosis of PMS was exhibited by the presence of cyclical manifestations of the disease in the luteal phase of menstrual cycle on the basis of history-taking and the results of patient's self-observation diary for 2-3 menstrual cycles (R. Moos Menstrual Distress Questionnaire). Mild PMS considered as the presence of 3-4 symptoms in 2-10 days before menstruation with significant severity 1-2 of them, severe form – the existence of 5-12 symptoms in 3-14 days before menstruation, 3-4 of them are most pronounced [7].

Inclusion criteria: reproductive age (18-44 years), regular menstrual cycle, presence of PMS, written consent from the patient. Exclusion criteria: pregnancy, lactation, disorders of menstrual cycle, focal lesions of breast, abnormal uterine bleeding of unknown etiology, acute inflammation of pelvic organs, tumors of uterus and ovaries of unknown etiology, endometrial hyperplasia, genital endometriosis, severe somatic pathology in the history, organic pathology of the central nervous system, mental illness, hormonal tumors, diabetes mellitus, adrenal diseases, thyroid pathology, malignant tumors in the present or in anamnesis, premenstrual dysphoric disorder, women who took psychotropic medications or hormonal therapy within the last three months.

Polymorphism of the genes was determined in the research laboratory of the Department of Medical Genetics, Shupyk National Medical Academy of Postgraduate Education (Kyiv). Material for the study was peripheral blood, which was collected into tubes with ethylenediaminetetraacetic acid in amount of 2.7 ml. Then deoxyribonucleic acid was extracted from samples of peripheral blood using commercial set "ДНК-коп6-В" (Central Research Institute of Epidemiology, Russia) according to the instruction. Polymerase chain reaction was done by "Fermentas" reagents (Lithuania) in the thermocycler «FlexCycler» (Analytik Jena, Germany). Restriction fragment length polymorphism was performed after polymerase chain reaction for study T-397C variant of ESR1 gene. The amplification products of the T-397C fragments were hydrolytically digested using the restriction endonuclease PvuII. The results of the restriction fragment length polymorphism were taken into account by conducting the electrophoresis of the resulting fragments in 2 % agarose gel (Fermentas agarose) with the addition of ethidium bromide and subsequent visualization using the Vitran computer system.

For statistical analysis we used program Statistica 6.0. We calculated arithmetic mean value, average standard error, criterion χ^2 (Yates corrected Chi-square), odds ratio (OR), confidence interval (CI). The nonparametric Mann-Whitney test was used to compare two independent groups by a single feature. The difference between the values that were compared was considered significant by $p \leq 0.05$.

RESULTS

The average age of the persons in the control group was 27.16 ± 1.09 years, the basic one – 29.42 ± 0.84 years. Menarche started approximately in equal age of women in both groups – 13.04 ± 0.20 and 12.94 ± 0.12 years respectively. The rate of gynecological pathology was also high in two groups – 21 (84.0 %) controls and 46 (92.0 %) patients with PMS. Most of the persons in the basic group had the combination of two-three gynecological diseases in anamnesis. Thus, inflammatory processes of appendages were diagnosed in 1.4 times more often (42 persons (84.0 %)) than in healthy individuals (15 women (60.0 %); $\chi^2=4.03$, $p=0.045$; OR=3.50, 95% CI 1.16-10.52, $p=0.03$), especially

in patients with severe PMS (22 women (88.0 %), $\chi^2=3.74$, $p=0.053$; OR=4.89, 95% CI 1.15-20.79, $p=0.03$). In history the inflammatory diseases of low genital tract had 11 (44.0 %) controls and 23 (46.0 %) women in the basic group, ovarian cysts – 3 (12.0 %) and 5 (10.0 %) persons respectively, disorders of menstrual cycle – 7 (28.0 %) and 15 (30.0 %), infertility – 1 (4.0 %) and 1 (2.0 %), gynecological operations – 2 (8.0 %) and 3 (6.0 %).

Reproductive anamnesis in controls differs from women with PMS. Only 11 (44.0 %) healthy persons were pregnant versus 35 (70.0 %) patients with PMS ($\chi^2=3.72$, $p=0.053$; OR=2.97, 95% CI 1.09-8.03, $p=0.03$). 10 (40.0 %) of controls had labors versus 32 (64.0 %) in basic group ($\chi^2=2.98$, $p=0.08$; OR=2.67, 95% CI 0.99-7.15, $p=0.05$). There was no significant difference in above mentioned indices between women with mild and severe forms of PMS.

The onset of the first premenstrual symptoms was at the similar age period in both PMS groups – 22.88 ± 0.99 years in patients with mild form and 23.04 ± 1.12 years – with severe one. Most of the clinical manifestations in women with PMS were connected with psychological (emotional lability, irritability, arousal) and physical disorders (peripheral edema, mastalgia, pain in bones, muscles, lumbalgia). It worth to mention, that all women with PMS declared that manifestations in the second phase of menstrual cycle reduced their daily activities.

Individuals with TC genotype of T-397C polymorphic variant of ESR1 gene were prevalent both in control and basic groups, accounting for about half of all the persons – 52.0 % in control group and 46.0 % in basic one ($\chi^2=0.06$, $p=0.81$; OR=0.79, 95 % CI 0.30-2.06, $p=0.62$) (table 1). The equal distribution of CC and TT genotypes also was determined between persons without (24.0 % and 24.0 % respectively) and with PMS (30.0 % and 24.0 % respectively). There was no significant difference in the rate of carriers of T and C alleles between persons with pathology (70.0 % and 76.0 % respectively) and without it (76.0 % and 76.0 % respectively). We did not find also significant difference in the frequency of genotypes and alleles between patients with mild and severe forms of the disease. The statistical results of data did not demonstrate the increased risk of T-397C polymorphic variant of ESR1 gene in the development of PMS (table 2).

DISCUSSION

One of the main pathogenetic mechanisms of PMS development is connected with changes of estrogens and progesterone levels during the menstrual cycle. Estrogens act through the α or β receptors which are genetically encoded by estrogen receptor genes ESR1 and ESR2. The research data about the study of the polymorphism of ESR gene demonstrated its role in the genesis of different pathological diseases. There are reports about its significance as the risk factor in the forming of cancer conditions – cancer of breast [8], endometrium [9], prostate [10], hepatocellular carcinoma [11], etc. However, the meta-analysis in EMBASE, PubMed, CNKI, and

Table I. The frequency of genotypes and alleles of T-397C variant of ESR1 gene in women with premenstrual syndrome.

Genotype / allele	Control group, n=25	Mild PMS, n=25	Severe PMS, n=25	PMS, total, n=50
TT genotype	6 (24.0)	4 (16.0)	8 (32.0)	12 (24.0)
TC genotype	13 (52.0)	15 (60.0)	8 (32.0)	23 (46.0)
CC genotype	6 (24.0)	6 (24.0)	9 (36.0)	15 (30.0)
T allele	19 (76.0)	19 (76.0)	16 (64.0)	35 (70.0)
C allele	19 (76.0)	21 (84.0)	17 (68.0)	38 (76.0)

Table II. Genotypes and alleles of T-397C variant of ESR1 gene as risk factors of premenstrual syndrome.

Genotype/allele	Indices	Mild PMS, n=25	Severe PMS, n=25	PMS, total, n=50
TT genotype	OR	0.60	1.49	1.00
	CI	0.15-2.47	0.43-5.17	0.32-3.08
	p	0.48	0.53	1.00
TC genotype	OR	1.38	0.43	0.79
	CI	0.45-4.25	0.14-1.37	0.30-2.06
	p	0.57	0.16	0.62
CC genotype	OR	1.00	1.78	1.36
	CI	0.27-3.67	0.52-6.09	0.45-4.07
	p	1.00	0.36	0.59
T allele	OR	1.00	0.56	0.74
	CI	0.27-3.67	0.16-1.92	0.25-2.21
	p	1.00	0.36	0.59
C allele	OR	1.66	0.67	1.00
	CI	0.41-6.78	0.19-2.33	0.32-3.08
	p	0.48	0.53	1.00

WANFANG systems, in which the literature data were collected till 2018, suggest that ESR1PvuII (rs2234693 T>C) polymorphism may only have little impact on cancer susceptibility [12].

Tang L. et al. studied that persons who have ESR1 PvuII T allele are in the risk group of hip fracture, while the XbaI polymorphism is not associated with it [13]. Knee osteoarthritis [14], osteoporosis [15] are connected with ESR1 gene polymorphism. The carriers of TC or CC and rs9340799-AG or GG genotype have the highest knee osteoarthritis risk, compared to individuals with rs2228480-TT and rs9340799-AA genotype [16].

PvuII and XbaI polymorphisms are associated with premature ovarian rupture [17], while other researches demonstrated opposite results [18]. The risk of infertility can be related to XbaI and PvuII polymorphisms of ESR1 in Pakistan population in persons with AG genotype and TC genotype [19]. GG genotype of XbaI polymorphism decrease the risk of preeclampsia, while homozygous T-A haplotype carriers of ESR1 PvuII and XbaI polymorphisms have increased risk [20]. Unexplained spontaneous abortions can be connected with ESR1 polymorphism [21]. There was no the statistically difference in the frequency distribution of the XbaI and of the PvuII genotypes and alleles between the persons with of central precocious puberty and controls. [22].

Also, the meta-analysis reported that PvuII (C>T) polymorphism was not related to the susceptibility to endometriosis except for a slight association of stage I–III endometriosis under recessive model. This meta-analysis indicated that the PvuII and XbaI polymorphisms were not associated with the risk of endometriosis [23]. Results of meta-analysis which studied the relation between ESR1 gene polymorphism (-351A/G and -397T/C variants) demonstrated the increased risk of severe preeclampsia in the carriers of GG genotype (XbaI -351A/G polymorphism) and no association of the ESR1 gene PvuII -397T/C and XbaI -351A/G polymorphisms with severe and mild preeclampsia [24].

Researches about ESR1 polymorphism as the risk factor of PMS are limited. They mostly are connected with the study of A-351G polymorphic variant. The equal frequency of A/G genotypes between women with and without PMS was found [25, 26]. Individuals with GG genotype have affective emotional features [25].

CONCLUSIONS

The study of rate of T-397C variants of estrogen receptor gene ESR1 in patients with PMS was first conducted. There is no association of T-397C polymorphic variant of estrogen receptor gene ESR1 with PMS.

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ORIGINAL ARTICLE

PERFORMANCE STATUS AND QUALITY OF LIFE AFTER RECONSTRUCTIONS OF BUCCAL MUCOSAL AND RETROMOLAR TRIGONE DEFECTS BY SKIN AND FASCIAL FLAPS IN ONCOLOGICAL PATIENTS

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ABSTRACT

The aim: of the study is to compare surgical and functional results as well as quality of life after use of skin flaps – platysma myocutaneous flap, supraclavicular artery flap and fascial flaps – temporoparietal fascial flap, buccal fat pad for plastic closure of the buccal and retromolar trigone mucous surgical defects in cancer patients.

Material and methods: A retrospective comparative analysis of the results of surgical treatment of buccal and retromolar trigone mucosal cancer in 56 patients operated from 2009 to 2014 was made. The patients were divided into two groups: the first included 26 patients, with reconstruction of fascial flaps, the second group included 30 patients, with reconstruction of postoperative defects with skin flaps. Surgical results: the average duration of surgery, the average duration of inpatient treatment and the presence of complications were compared in the both groups. Functional results in both groups were compared in 6 and 12 months after surgery with the Performance Status Scale for Head and Neck Cancer Patients (PSS-HN), and quality of life was assessed by the University of Washington Quality of Life questionnaire, version 4.

Results: Average duration of surgery, average time of inpatient treatment, and postoperative complications did not differ significantly in both groups ($P > 0.05$). Data of performance status and quality of life in patients with reconstruction of mucosal defects and retromolar trigone with skin flaps were significantly better ($P > 0.05$) in 6 and 12 months after surgery, compared with the group of patients who underwent defect reconstruction with fascial flaps.

Conclusions: The method of choice in reconstruction of medium and large defects of the buccal and the retromolar trigone mucous is usage of skin flaps: the platysma myocutaneous flap and the flap of the supraclavicular artery.

KEY WORDS: buccal mucosal cancer, reconstruction of mucous defects, skin and fascial flaps, performance status, quality of life

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INTRODUCTION

Oral cancer is the sixth most common malignancy worldwide [1]. Three hundred thousand patients (2.1% of the total cancer cases) were afflicted with cancer of the oral cavity and lip in 2012 [2]. Cancer of the buccal mucous and retromolar trigone in the oriental countries accounts up to 20% of all malignant neoplasms of the oral cavity and is characterized by an aggressive course and a high frequency of recurrence [3]. Treatment of patients with locally advanced mucosal cancer involves surgery with single-stage reconstruction and adjuvant radiotherapy or concomitant chemoradiotherapy [4]. Having removed a malignant tumor of the buccal mucous, a buccal defect appears, which involves several layers of the buccal, rarely – all layers, including the skin. Buccal defects can be combined with marginal or segmental defect of the mandible or partial defect of the

upper jaw [5]. Ensuring good functional and aesthetic results are crucial in the reconstruction of the buccal after oncologic resection [6].

Depending on postoperative defects of the buccal mucous size after removal of the primary tumor, they are divided into three types: small (up to 3 cm in the largest dimension); medium (3 to 6 cm in the largest dimension); large (larger than 6 cm in the largest dimension). Small defects can be closed with local tissues, while medium and large defects require plastic replacement using local, regional, or free flaps.

There are no clear recommendations in the literature regarding the choice of reconstructive technique for replacement of buccal mucosal defects. For plastic replacement of medium defects of the buccal they use a buccal fat pad (BFP) and platysma myocutaneous flap (PMF) [7,8]. Major defects are closed with regional supraclavicular

Table I. Characteristics of the studied patients

Characteristics Abs.		FF group (BFP, TPFF) (n=26)		SF group (SAF, PMF) (n=30)		p-value
		%	Abs.	%		
Sex	female	5	19,2	6	20,0	0,79
	male	21	80,8	24	80,0	
Subsite of oral cavity	Retromolar trigone	10	38,5	8	26,7	0,51
	Buccal mucosa	16	61,5	22	73,3	
T staging	T2	6	23,1	3	10,0	0,39
	T3	17	65,4	22	73,3	
	T4	3	11,5	5	16,7	
N staging	N0	9	34,6	13	43,3	0,75
	N1	9	34,6	10	33,3	
	N2	8	30,8	7	23,4	
M staging	M0	26	100	30	100	>0,99
Clinical stage	III	16	61,5	20	66,7	0,91
	IV	10	38,5	10	33,3	

Note. FF – fascial flaps, SF – skin flaps, BFP – buccal fat pad, TPFF – temporoparietal fascial flap, SAF – supraclavicular artery flap, PMF – platysma myocutaneus flap. Comparison of groups in accordance to Chi-square.

artery flap (SAF) and the temporoparietal fascial flap (TPFF) [9,10].

One of the key factors determining the functional results of plastic replacement of medium and large mucous defects in cancer patients is the preservation of the possibility of mouth opening, which is greatly affected by scarring and the formation of coarse connective tissue at the site of plastic closure. It depends on the presence of epithelial skin lining on the part of the flap that directly closes the buccal mucous defect.

To close the buccal mucous defect may be used fascial flap without epithelial skin lining – temporoparietal fascial flap (TPFF) and buccal fat pad (BFP), as well as flap with epithelial skin lining – platysma myocutaneus flap (PMF) and supraclavicular artery flap (SAF).

In the available literature we did not find any data according to the significance of the presence of skin epithelial lining in regional and local flaps used to close the middle and large defects of the buccal mucous for functional results and quality of life in the postoperative period.

THE AIM

The aim of the work is to compare surgical and performance status and quality of life after use of skin flaps (SAF and PMF) and fascial flaps (BFP and TPFF) in cancer patients for plastic closure of mucosal defects.

MATERIALS AND METHODS

A retrospective comparative analysis of the surgery results was made in 56 patients with cancer of the buccal mucous

and retromolar trigone, who were treated at the *Head and Neck Oncology Department* in the National Cancer Institute during the period since 2009 to 2014. Patients were divided into two groups: the first included 26 patients who underwent reconstruction of postoperative defects with fascial flaps: BFP – 10 patients and TPFF – 16 patients; second group included 30 patients who underwent reconstruction of postoperative defects with skin flaps: SAF – 19 patients and PMF – 11 patients.

There were 11 (19.6%) women and 45 (80.4%) men among all patients, the average age of patients was 56.0 ± 8.1 years (from 38 years to 76 years). The average age of patients with fascial flaps was 54.8 ± 7.8 years, the average age of those with skin flaps was 57.1 ± 8.4 years (the difference was not statistically significant, $p = 0.29$).

A squamous cell carcinoma of low grade (G1) returned in 15 patients, moderate grade (G2) in 30 patients and high grade (G3) in 11 patients. It was histologically confirmed prior to treatment.

In accordance to process spread stage III (T2N1M0, T3N0-1M0) was diagnosed in 36 patients (61.5% and 66.7% respectively), stage IV (T2-3N2M0, T4N1-2M0) was diagnosed in 20 patients (38.5% and 33, 3% respectively). The characteristics of the studied patients are presented in Table 1.

Having made the analysis no differences were found between the groups neither by sex, localization, stage, or TNM ($p > 0.05$).

All patients were underwent surgical treatment in the volume of resection of the buccal and/or retromolar trigone, cervical lymph node dissection and simultaneous

Table II. Post-operative complications after reconstruction of defects of the buccal mucosa and retromolar trigone with regional skin and fascial flaps

Complication	FF group (BFP, TPFF), (n=26)	SF group (SAF, PMF), (n=30)	p-value
Flap complication			
Flap failure	0	1 (3,3 %)	>0,99
Partial flap necrosis	0	2 (6,7 %)	
Marginal flap necrosis	2 (7,7 %)	0	
Total	2 (7,7 %)	2 (10 %)	
Recipient site complication			
Salivary fistula	0	2 (6,7 %)	>0,99
Orostoma	0	0	
Haematoma	2 (7,7 %)	0	
Infection complication	1 (3,8 %)	2 (6,7 %)	
Wound dehiscence	0	0	
Total	3 (11,5 %)	4 (13,3 %)	
Donor site complication			
Focal alopecia	8 (30,7 %)	NA	0,047
Damage to the frontal branch of the facial nerve	0	0	
Damage to the marginal branch of the facial nerve	0	0	
Cheek deformity	1 (3,9%)	0	
Wound dehiscence	0	3 (10 %)	
Impairment of shoulder function	0	0	
Total	9 (34,6 %)	3 (10 %)	

Note. NA – not applicable

Group comparisons were performed by Fisher's exact test.

plastic replacement of the defect. Buccal reconstruction was performed simultaneously with the removal of the primary tumor and regional lymph nodes.

The patients were included in the study by the criteria: locally advanced mucosal cancer without prior radiotherapy or chemotherapy; plastic replacement of the middle or large defects of the buccal alone or combined with the mandibular or maxillary defects after the removal of the primary tumor.

Criteria not to include the patients in the study were: defects of the buccal mucous combined with a segmental defect of the mandible; perforated buccal defects; decompensated heart failure; diabetes with unstable hyperglycemia; the period of 3 months after a heart attack or stroke; when the patient's condition was defined as a contraindication to reconstructive surgery.

In both groups, comparison of the early results of flaps use for the reconstruction of the buccal with and without the skin pad, the features of surgical technique, the average duration of surgery, the average duration of inpatient treatment and the presence of complications was made.

Late functional results of using flaps with and without the skin pad were compared in 6 and 12 months after surgery, basing on the Performance Status Scale for Head and Neck Cancer Patients (PSS-HN). Quality of life was assessed by the University of Washington Quality of Life questionnaire, version 4 (UW-QOL v4).

Statistical analysis of the study results was carried out in the package MedCalc v. 18.11 (MedCalc Software bvba, Belgium, 1993-2018).

The mean value ($\bar{X}$) and its standard deviation ($\pm$ SD) were calculated to represent the quantitative traits, and the trait frequency (%) for the qualitative traits. When comparing quantitative traits, the Student's criterion (in the case of a normal distribution law) or the W-Wilcoxon criterion (in the case of a distribution law other than the normal one) were used, and the normality distribution test was performed using the Shapiro-Wilk test. The Chi-square test (with Yeats' correction) was used to compare qualitative indicators. To evaluate the clinical effect, its magnitude and 95% probable interval (95% CI) were calculated. The critical level of significance is assumed to be $\alpha = 0.05$.

RESULTS

The average duration of the reconstructive surgery with skin flaps was 4.3 ± 0.7 h, it didn't significantly differ ($P = 0.88$) from the reconstructive surgery with fascial flaps, which lasted 4.2 ± 0.8 h. The mean duration of inpatient treatment in the group of patients with fascial flaps was 10.3 ± 2.1 days, it didn't significantly differ ($P = 0.48$) from the group of patients with skin flaps, in which the average length of stay in hospital was $10.7 \pm 2,1$ days.

Table III. Performance status of Head and Neck (PSS-HN) after 6 and 12 months after surgery in patients with reconstruction of defects of the buccal mucosa and retromolar trigone by regional skin and fascial flaps

PSS-HN	6 month after surgery			12 month after surgery		
	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value
	Mean $\pm$ SD	Mean $\pm$ SD		Mean $\pm$ SD	Mean $\pm$ SD	
Normalcy of diet	58,8 $\pm$ 15,8	68,3 $\pm$ 14,6	0,004	61,7 $\pm$ 15,0	74,1 $\pm$ 14,2	0,007
Eating in public	70,2 $\pm$ 17,3	77,5 $\pm$ 7,6	0,04	69,6 $\pm$ 18,3	80,5 $\pm$ 11,2	0,06
Understandability of speech	86,5 $\pm$ 12,7	87,5 $\pm$ 12,7	0,78	89,1 $\pm$ 12,7	91,7 $\pm$ 12,0	0,81

Note. W-Wilcoxon test was used for comparison: the significant difference is in bold.

Table IV. Quality of life according to the UW-QOL v4 questionnaire 6 and 12 months after surgery in patients with reconstruction of buccal mucosa and retromolar trigone with regional skin and fascial flaps

UW-QOL v4	6 month after surgery			12 month after surgery		
	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value	FF group (BFP, TPFF), %, (n=26)	SF group (SAF, PMF), %, (n=30)	p-value
	$\bar{X} \pm SD$	$\bar{X} \pm SD$		$\bar{X} \pm SD$	$\bar{X} \pm SD$	
Pain	75,0 $\pm$ 20,0	81,7 $\pm$ 18,5	0,21	76,1 $\pm$ 19,2	84,3 $\pm$ 18,5	0,12
Appearance	71,2 $\pm$ 26,2	80,8 $\pm$ 10,8	0,24	72,8 $\pm$ 27,1	82,4 $\pm$ 11,6	0,37
Activity	78,8 $\pm$ 18,3	81,7 $\pm$ 17,3	0,57	83,7 $\pm$ 16,2	85,2 $\pm$ 15,9	0,74
Recreation	76,0 $\pm$ 16,6	77,5 $\pm$ 17,8	0,72	79,3 $\pm$ 19,4	81,5 $\pm$ 16,4	0,75
Swallowing	95,2 $\pm$ 10,0	97,5 $\pm$ 7,6	0,33	97,8 $\pm$ 7,2	97,2 $\pm$ 8	0,78
Chewing	53,8 $\pm$ 28,0	70,0 $\pm$ 24,9	0,03	52,2 $\pm$ 31,9	72,2 $\pm$ 25,3	0,03
Speech	91,9 $\pm$ 13,6	92,0 $\pm$ 13,5	0,98	96,1 $\pm$ 10,3	93,3 $\pm$ 12,7	0,4
Shoulder	83,8 $\pm$ 15,3	77,0 $\pm$ 12,9	0,07	80 $\pm$ 18,3	75,6 $\pm$ 11,9	0,2
Taste	57,7 $\pm$ 18,8	51,3 $\pm$ 20,3	0,23	67,8 $\pm$ 13,5	68,1 $\pm$ 12,4*	0,95
Saliva	46,9 $\pm$ 20,2	47,3 $\pm$ 20,2	0,94	66,5 $\pm$ 11,5*	65,6 $\pm$ 12,8*	0,78
Mood	66,3 $\pm$ 17,2	72,5 $\pm$ 15,2	0,13	66,3 $\pm$ 14,3	73,1 $\pm$ 16,9	0,15
Anxiety	50,8 $\pm$ 24,2	68,3 $\pm$ 20,7	0,006	55,2 $\pm$ 24,1	75,9 $\pm$ 19,1	0,002
General 1	50,0 $\pm$ 15,8	56,7 $\pm$ 17,3	0,13	51,1 $\pm$ 17,6	57,4 $\pm$ 11,6	0,19
General 2	53,1 $\pm$ 13,8	58,7 $\pm$ 12,8	0,14	53,9 $\pm$ 15,3	58,5 $\pm$ 12,3	0,26
General 3	55,4 $\pm$ 13,0	62,0 $\pm$ 16,1	0,12	56,5 $\pm$ 15,6	63 $\pm$ 15,4	0,16

Note. W-Wilcoxon test was used for comparison: the significant difference is in bold.

* - the difference from the corresponding indicator in 6 and 12 months. after surgery, statistically significant, $p < 0.05$.

The analysis of postoperative complications in both groups included the assessment of complications in the transplanted flap, in the postoperative wound and in the donor site (Table 2). We did not observe significant differences in the transplanted flap complications in both groups (7.7% vs 10%, $p > 0.99$) and in the postoperative wound (11.5% vs 13.3%, $p > 0.99$). At the same time, complications in the donor site in the group of patients with flap reconstruction without skin pad were significantly greater comparing with the skin flaps group (34.6% vs 10%, $p = 0.047$), due to Focal alopecia observed in 8 patients, representing 30.7% of the RFF group after using TPFF.

Significantly better data of diet completeness (58,8 $\pm$ 15,8% vs 68,3 $\pm$ 14,6%, $p = 0,004$) and nutrition in public

(70.2 $\pm$ 17.3% vs 77.5 $\pm$ 7.6%, $p = 0.04$) in accordance to the PSS-HN scale were observed in the group of patients with skin flaps reconstruction after 6 months. In 12 months the Normalcy of diet in accordance to the PSS-HN scale was significantly better ($p = 0.007$) in the RSF group. Understandability of speech in both groups did not differ significantly (Table 3) in 6 months ($p = 0.78$) and 12 months after surgery ($p = 0.81$).

In both groups comparing the quality of life data (Table 4) in accordance to the scale UW-QOL v4 it was found that in the RSF group Chewing was significantly better than the RFF group after 6 months (70.0 $\pm$ 24.9% vs 53.8 $\pm$ 28.0%, $p = 0.03$) and 12 months (72.2 $\pm$ 25.3% vs 52.2 $\pm$ 31.9%, $p = 0.03$) after surgery.

In 6 and 12 months after surgery in both groups comparing the quality of life data (Table 4) in accordance to the UW-QOL v4 scale it was found that after 6 months ($70.0 \pm 24.9\%$ vs $53.8 \pm 28.0\%$, $p = 0.03$) and 12 months ($72.2 \pm 25.3\%$ vs $52.2 \pm 1.9\%$, $p = 0.03$). After surgery in the RSF group Chewing was significantly better than in the RFF group. Anxiety, like Chewing, significantly differed ($p < 0.05$) in both groups after 6 and 12 months after surgery.

Analyzing the dynamics of changes of the Saliva index in the RFF group (BFP, TPFF) an increase was revealed ($p < 0.05$) in 12 months ($66.5 \pm 1.5\%$) comparing with its value in 6 months ($46.9 \pm 20.2\%$) after surgery. Similar changes in the Saliva index were estimated in the RSF group in 6 and 12 months (SAF, PMCF), $p < 0.05$. Within 12 months, a significant improvement in the Taste index ($68.1 \pm 12.4\%$ vs $51.3 \pm 20.3\%$, $p < 0.05$) was found after 12 months compared to 6 months. In the RFF group, the change in the Taste index after 6 and 12 months was insignificant ($67.8 \pm 13.5\%$ vs $57.7 \pm 18.8\%$, $p > 0.05$). Comparing other data of quality of life, no significant difference was found in both groups in 6 and 12 months after surgery ($p > 0.05$).

DISCUSSIONS

The experience of plastic replacement of postoperative mucosal defects as well as the analysis of the oncological results of treatment of patients with squamous cell carcinoma of the buccal mucous are based on studies with a small amount of clinical observations [11]. We hope, that our study will complete the data of literature, including the study of the performance status and quality of life in patients with skin and facial flaps to replace the defects of the buccal mucous and retromolar trigone in cancer patients.

The morbidity of squamous cell carcinoma of the buccal mucosa in Europe and North America is only 10% of all malignant neoplasms of the oral cavity. In Taiwan [1], mucosal cancer is a much more common disease, but a little research has been done to investigate various reconstructive approaches to correct mucosal defects.

We obtained the results of replacement of medium and large defects of the buccal mucous with regional or local flaps and related data: features of surgical technique, the average duration of surgery, the average length of stay in the hospital, the presence of complications in the transplanted flaps and in the recipient wound, which showed no differences between groups.

A small number of complications in the transplanted flap, in particular the supraclavicular artery flap (we did not observe cases of total necrosis of SAF), are associated with the refinement of surgical technique, in particular the flap marking was performed according to the course of the supraclavicular artery, which was determined by Doppler ultrasound. Described by Padiyar and co-authors [9], total flap failure of SAF in 28% of cases is associated with flap marking by anatomical landmarks. Therefore, in our opinion, it is the performance status and quality of life of the patients that are the key criteria for the choice of reconstruction method for the replacement of medium and large defects of the buccal mucous in cancer patients.

In the group of patients who underwent reconstruction with fascial flaps, our data confirm data of D. Hwang [7] that use of BFP may cause development of post-operative trismus and related functional disorders as well as deterioration of quality of life after plastic closure of small size defects, less than 6 cm. In the closure of small defects in the group of patients with skin flaps, among which we most often used PMF the data obtained by us are similar to the results obtained by L. Huong.

In closure of large defects of the buccal mucous (more than 6 cm) in the group of patients with fascial flaps we most often used TPFF, and in the group of patients with a skin pad flaps we most often used SAF. We found that the use of SAF in comparison with TPFF to replace major mucosal defects provides significantly higher normalcy of diet and nutrition in public in accordance to the PSS-HN functioning scale as well as data of chewing, anxiety in accordance to the UW-QOL quality of life questionnaire. In our opinion, lower values of Chewing, Normalcy of diet and nutrition in public in the case of TPFF, as well as in the case of BFP are conditioned by fibrotic buccal changes and trismus, which led to impaired chewing function. Patients are less concerned in case of SAF due to the fact that postoperative scar on the shoulder is more easily perceived by patients than the scar in the temporal and parietal region, which is often accompanied by Focal alopecia.

A promising direction for further research is to compare the results of replacement of medium and large mucosal defects with the regional, local and free flaps.

CONCLUSIONS

1. There were no statistically significant differences in the duration of surgery, inpatient treatment, the incidence of complications after flap transplantation, postoperative complications between groups of patients whose medium and large defects of the buccal mucous and the retromolar trigone were replaced by skin and fascial flaps.
2. The use of skin flaps to replace the medium and large defects of the buccal mucous significantly improves the performance status of the diet normalcy and nutrition in public and the quality of life of patients in terms of chewing, anxiety compared with the use of fascial flaps without skin pad.
3. In the reconstruction of medium and large defects of the buccal mucous the method of choice is the use of skin flaps – platysma myocutaneu flap (PMF) and regional flap of the supraclavicular artery (SAF).

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ORIGINAL ARTICLE

SPECIAL ASPECTS OF THE EFFICIENT DAILY ROUTINE OF STUDENTS AT MODERN EDUCATIONAL ESTABLISHMENTS OF DIFFERENT TYPES

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ABSTRACT

The aim of study is special aspects of the efficient daily routine of students at modern educational institutions of different types, and its influence on psychophysical functions, adaptive abilities, the quality of life as well as the state of mental and somatic health of the youngsters.

Materials and methods: While studying special aspects of the daily routine of students at modern educational institutions and their influence on the state of physical and mental health, the young people were observed in five modern educational establishments in Ivano-Frankivsk: Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk Finance Commercial Cooperative College named after S.Granat, Ivano-Frankivsk music school named after Denis Sichinskiy, Ivano-Frankivsk Vocational Motor Transport and Construction Lyceum № 15, Ivano-Frankivsk boarding lyceum for gifted young people from rural areas. 300 students were observed (150 female students and 150 male students). The study analyzed their educational conditions, daily routine tension, and activities during breaks, special aspects of leisure arrangements, amount of time spent on homework, subjective assessment of the level of tension of the educational process, and their projected influence on the health state of students by using static models.

Results: Received results of the assessment of sanitary and hygienic conditions under which training takes place at modern educational institutions of different types, as well as conditions of after school activities of the students, by applying common methods to evaluate illumination level, microclimate, and air quality have revealed that the youngsters' conditions at modern educational institutions are marked by absence of significant deviations from common hygienic requirements, they are similar and easily comparable and that enables defensibly and illuminatively to identify main features of influence of the different psychohygienic methods on the development of health maintaining tools and the establishment of features of the formation of functional capabilities and the basic correlates of mental and physical health.

Conclusions: The educational conditions of young people in modern educational establishments can be described as not having considerable deviations from common hygienic requirements, being easily comparable and enabling to identify main influence factors of different types of psychohygienic methods of developing and implementing of health-maintaining tools on the course of formation of the functional capabilities of the body and the development of the main adaptation reactions in young males and females.

KEY WORDS: daily routine, educational process, educational institutions, students, health status

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INTRODUCTION

According to the data of the World Health Organization, health assessment of the Ukrainian population in the present-day conditions appears to be not merely a medical issue but a complex matter. The formation of health culture guarantees self-fulfillment of the youth, providing active leisure, possibilities to start a family, fulfillment of hard training and work, social and political activities. Young people in the modern society often make health their first priority but spare no effort to maintain and improve it. Therefore, we should pay a great attention to teaching methods of promoting healthy lifestyle behaviors among students and building health value. Need for health has to become a standard of human life. While analyzing scientific resources, two main notions stand out: "culture" and "health". The notion "culture" covers science, education, ethics and life style [1-3]. "Health" is affected by the factors, which could be split into 2 groups. The first group includes factual characteristics of the educational process: duration of the school day, study load, activities during

the breaks, microclimate in rooms. The second group consists of subjective personal characteristics: diet, sport and morning exercises, hardening, leisure activities, and bad habits. Under real conditions, just the second group of factors really influences the health of young people. Therefore, we should constantly promote ideas of healthy lifestyle, develop skills of healthy lifestyle, and engage into establishment of principles of healthy lifestyle. Health of a person mainly depends on their lifestyle, working conditions, physical activity, leisure aspects, diet and habits.

Daily routine and leisure time are significant for recovery. Daily routine is an assigned order, duration and alteration of work and rest during a day, as well as diet, sport, morning exercises, sleep, observing rules of personal hygiene. It is a general pace of life and activities of students, which takes into consideration biological rhythms, as well as special aspects of physical and mental load. Regular change of the routine elements sets certain rhythm of body processes and creates a reflex, which can last for some time. A daily routine facilitates setting of certain biological rhythms of a body. [4, 5].

A certain duration of physical or mental work, an alteration of different activities, a balanced diet, a necessary duration of the night sleep, regular morning exercises and enough movement provide conditions to improve working efficiency and successful personal development of students

The main feature of the healthy lifestyle of students at modern educational establishments of different types is an efficient daily routine, which includes well-organized studying, a healthy diet, physical activity, morning exercises, body strengthening procedures and absence of bad habits. The basic objective of the efficient daily routine is to create condition for efficient physical and mental work of students and the most comfortable functioning of all body systems. The fundamental regulation tool is alteration of different activities during a day.

THE AIM

The study aims is special aspects of the efficient daily routine of students at modern educational institutions of different types, and its influence on psychophysical functions, adaptive abilities, the quality of life as well as the state of mental and somatic health of the youngsters.

MATERIALS AND METHODS

While studying special aspects of the daily routine of students at modern educational institutions and their influence on the state of physical and mental health, the young people were observed in five modern educational establishments in Ivano-Frankivsk: Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk Finance Commercial Cooperative College named after S. Granat, Ivano-Frankivsk music school named after Denis Sichinskiy, Ivano-Frankivsk Vocational Motor Transport and Construction Lyceum No 15, Ivano-Frankivsk boarding lyceum for gifted young people from rural areas. 300 students were observed (150 female students and 150 male students). The study analyzed their educational conditions, daily routine tension, and activities during breaks, special aspects of leisure arrangements, amount of time spent on homework, subjective assessment of the level of tension of the educational process, and their projected influence on the health state of students by using static models.

The selection of objects for observation, their grouping and classification according to the main indicators was performed considering the quantitative and qualitative representativeness of the research. The number of subjects surveyed in each group was estimated using mathematical formulas that determined the minimum amount of statistical sample and obtaining reliable results in accordance with the objectives and determined the quantitative representativeness of the research results, which guarantees the validity of the work.

In order to achieve the homogeneity of the studied groups and to ensure the necessary level of qualitative representativeness of the work, there were taken into consideration gender and age of the surveyed, psychological and pedagogical conditions of the educational environment, as well

as examining of the conditions of training and after school activities, duration of homework, activity during breaks, participation in sports sections, morning exercises and qualitative and quantitative indicators of the influence of the factors of the educational environment on the somatic health of young males and females. As part of the research process new modern highly informative hygiene, medical and sociological, epidemiological, psychological, physiological, psychodiagnostic and methods of multivariate statistical analysis and forecasting with strict observance of phasedness and sequence of their conduct were used. There was carried out a sanitary-hygienic assessment of the regime of the day and independent work, as well as studied the intensity of training, activity at a break, the way morning exercises are done, sports, recreation, housing and living conditions and the conditions of the educational environment of boys and girls.

Required data was obtained from specially designed questionnaires and interviews, also there was carried out hygienic assessment of the main components of the daily routine of students, and their health was examined too. The main characteristics of correlation between the state of health and the conditions in educational institutions, living and social conditions of life outside school, the daily routine and leisure, sports, strengthening, activity during breaks, intensity of the educational process of boys and girls were determined by methods of descriptive statistics and correlation analysis.

The statistical processing of the results of the study was carried out using programs of multivariate statistical analysis "Statistica 5.5 for Windows" (owned by the Center of new Information Technologies of the Vinnitsa National Medical University named after M.I. Pirogov, license No AXX910A374605FA) and "Statistica 6.0 for Windows" (ownership of the Center of new Information Technologies of the Vinnitsa National Medical University named after M.I. Pirogov, license #BXXR901E245722FA). An important role in the statistical processing of the obtained results belonged to the methods of descriptive statistics of data that characterized the formation and dynamic changes of the studied characteristics and were used to determine the arithmetic mean values (M), standard deviations (s) and standard errors of average values (m). The degree of validity of the data was carried out using parametric (calculation of the Student's criterion (t)) and non-parametric parameters (Pearson's (P_2) and Wilcoxon criteria (W)), and their results were evaluated according to the significance level (p). For an in-depth, practically-significant statistical evaluation of the comparison groups, the method of comparing the frequencies of the binary trait was used in two unrelated groups using a method of analysis of tables 2×2 . To assess the course of adaptation processes and predict their impact on the health of students correlation, cluster and factor analysis were applied.

RESULTS AND DISCUSSION

Received results of the assessment of sanitary and hygienic conditions under which training takes place at modern ed-

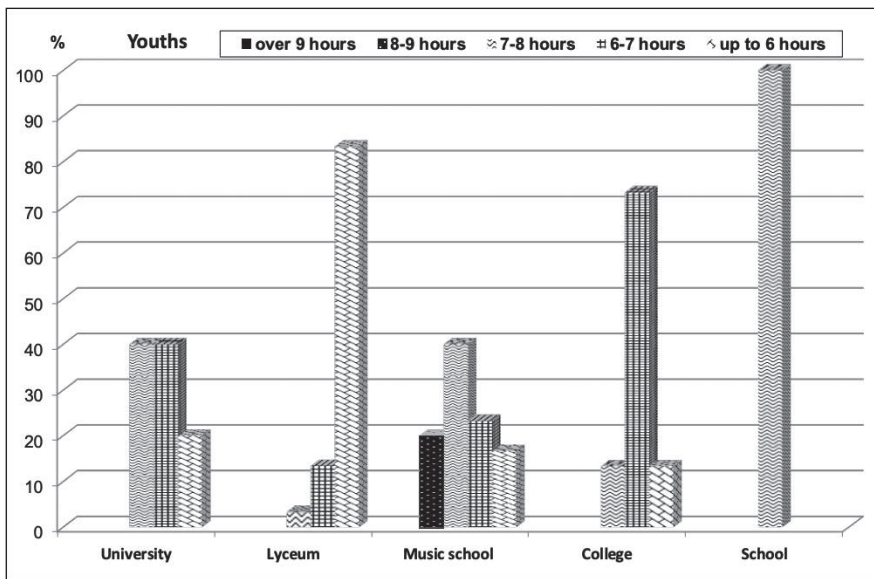


Fig. 1. Duration of stay in an educational institution, young male

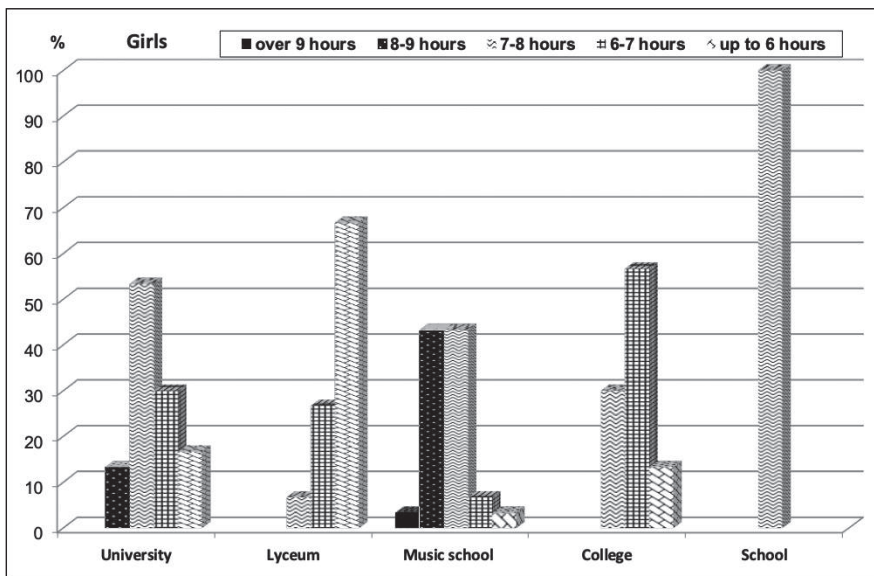


Fig. 2. Duration of stay in an educational institution, young female

educational institutions of different types, as well as conditions of after school activities of the students, by applying common methods to evaluate illumination level, microclimate, and air quality have revealed that the youngsters' conditions at modern educational institutions are marked by absence of significant deviations from common hygienic requirements, they are similar and easily comparable and that enables defensibly and illuminatively to identify main features of influence of the different psychohygienic methods on the development of health maintaining tools and the establishment of features of the formation of functional capabilities and the basic correlates of mental and physical health. [6].

While analyzing the time students spent in educational establishments, it should be emphasized that only 3.3% of girls in the college had more than 9 hours of training activity. Between 8 and 9 hours, this figure described 30.0% of boys and 13.3% of girls in the college and 43.3% of boys and 20.0% of girls in the music school.

Between 7 and 8 hours were spent by 53.3% of boys and 40.0% of girls at university, 73.3% of boys and 56.7% of girls

at the college, 40.0% of boys and 43.3% girls of the music school, 3, 3% of boys and 6.7% of girls in the lyceum, as well as 100.0% of boys and 100.0% of girls in the school respectively. The duration from 6 to 7 hours was for 40.0% of boys and 30.0% of girls of the university, 13.3% of boys and 13.3% of the college girls, 23.3% of boys and 6.7% girls of the music school and 13.3 % of boys and 26.7% of girls in lyceum. Up to 6 hours – for 20,0% of boys and 16,7% of girls of the university, 16,7% of boys and 3,3% girls of the school and 83,3% of boys and 66,7% of girls of lyceum (Fig. 1, Fig. 2).

The obtained results revealed the existence of clearly expressed signs of a significant excess of sanitary and hygiene requirements for the duration of daytime stay of students in various modern educational institutions, which has a significant negative impact on the level of functional and psychological capabilities of the body of students [7-9]. Indicators of the time students spent on homework assignments and the projects provided by the curriculum, on self-study assignments and home reading

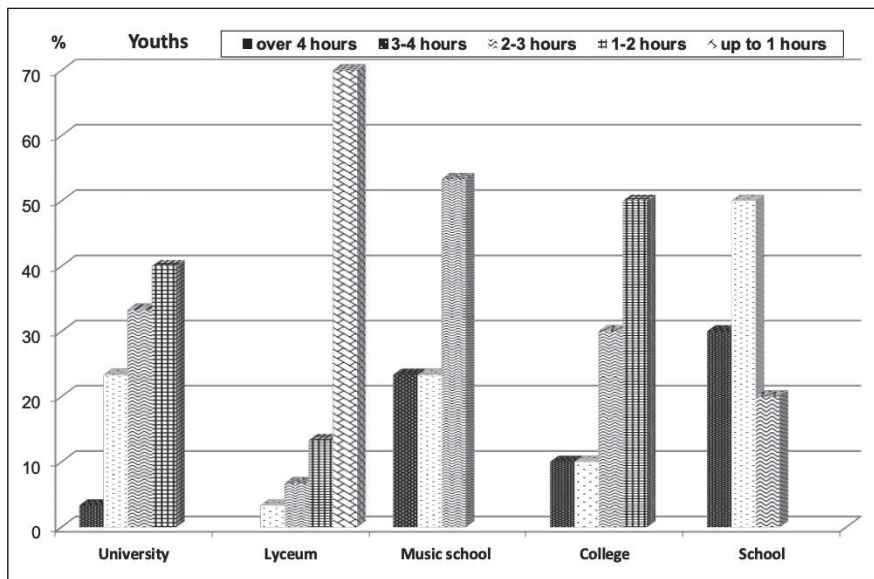


Fig. 3. Time spent on homework, young male

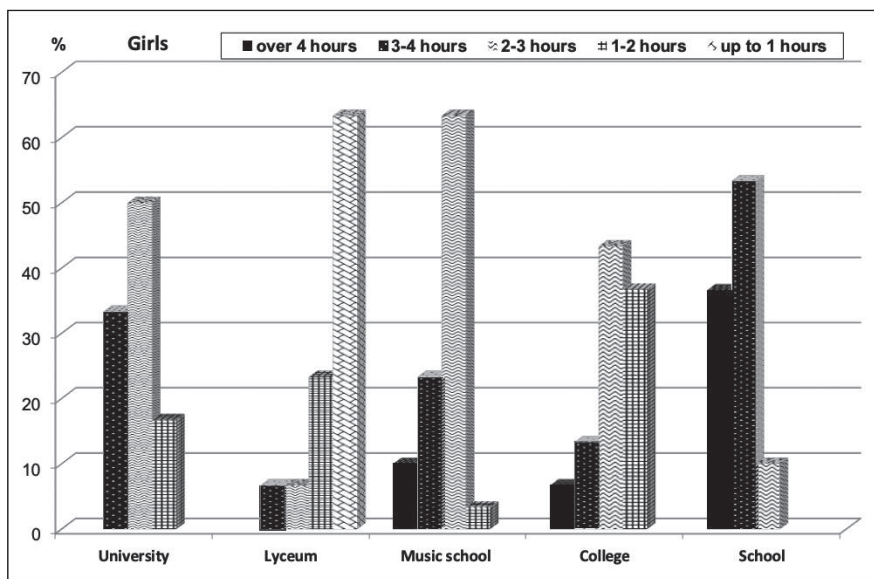


Fig. 4. Time spent on homework, young female

described the following time distribution (Fig. 3, Fig. 4). More than 4 hours on these activities were spent by 3.3% of university students, 10.0% of boys and 6.7% of college girls, 23.3% of boys and 10.0% of girls of music school, as well as 30.0% of boys and 36.7% of girls in school. From 3 to 4 hours – 23.3% of boys and 33.3% of university girls, 10.0% of boys and 13.3% of college girls, 23.3% of boys and 23.3% of girls of the musical school, 3.3% of boys and 6.7% of girls in the lyceum, as well as 50.0% of boys and 53.3% of girls in the school. From 2 to 3 hours, respectively, 33.3% of boys and 50.0% of university girls, 30.0% of boys and 43.3% of college girls, 53.3% of boys and 63.3% girls of the music school, 6.7% of boys and 6.7% girls in the lyceum, as well as 20.0% of boys and 10.0% of girls in the school. From 1 to 2 hours – 40.0% of boys and 16.7% of university girls, 50.0% of boys and 36.7% of college girls, 3.3% girls of the music school and 13.3% of boys and 23.3% of girls in the lyceum. Only students of the lyceum spent up to 1 hour on homework – 76.7% of boys and 63.3% of girls respectively.

While analyzing the scheduled breaks and studying the way they are arranged, it was established that they are organized in these educational institutions as standard, and students spend time during such breaks mostly passively. According to the results, passive leisure during breaks was typical for 86.7% of boys and 63.3% of girls of the university, 76.7% of boys and 46.7% of college girls, 76.7% of boys and 67.7% of girls at the music school, 80.0% boys and 70.0% girls of the lyceum, as well as 76.7% of boys and 33.3% of girls of the school. In general, during the scheduled breaks the following proportions of students were preparing for the following lessons: respectively 6.7% of boys and 20.0% of girls of the university, 16.7% of boys and 43.3% of college girls, 20.0% of boys and 20.0% of girls at the music school, 10.0% of boys and 3.3% of girls in the lyceum, as well as 20.0% of boys and 56.7% of girls in the school. Active forms of rest during breaks were characteristic of 6.7% of boys and 16.7% of university girls, 6.7% of boys and 10.0% of college girls, 3.3% of boys and 13.3% of girls of the music school, 10.0% of boys and 26.7% of girls in the lyceum, as well as 3.3% of boys and 10.0% of girls in the school. Most of the students in their free

time were watching television, that is 53.3% of boys and 50.0% of girls at the university, 73.3% of boys and 40.0% of college girls, 80.0% of boys and 50.0% girls of the music school, 53.3% of boys and 50.0% of girls of lyceum, as well as 73.3% of boys and 40.0% of girls of the school. Non-educational institutions were attended by 13.3% of boys and 20.0% of university girls, 6.7% of boys and 33.3% of college girls, 3.3% of boys and 10.0% of girls of the music school, 13.3% of boys and 20.0% of girls in the lyceum, as well as 6.7% of boys and 33.3% of girls in the school. 3.3% of boys and 3.3% of university girls, 6.7% of boys and 3.3% of college girls, 10.0% of boys and 23.3% girls of the music school, 3.3% of boys and 3.3% of girls in the lyceum, as well as 3.3% of boys and 3.3% of girls in the school work in their degree field or have other part-time jobs.

The prospects for further research can be in the further comprehensive study of the peculiarities of the daily routine of students at modern educational institutions of different types, the assessment of the impact of the daily routine on adaptive capacity, quality of life and the state of physical and mental health of boys and girls.

CONCLUSIONS

1. The educational conditions of young people in modern educational establishments can be described as not having considerable deviations from common hygienic requirements, being easily comparable and enabling to identify main influence factors of different types of psychohygienic methods of developing and implementing of health-maintaining tools on the course of formation of the functional capabilities of the body and the development of the main adaptation reactions in young males and females.
2. Indicators of the assessment of the characteristics of the basic social and household characteristics of the organization of life and the educational environment of students show the existence of favorable housing, living conditions and conditions of permanent residence in the vast majority.
3. While assessing the daily routine of students at modern educational institutions, special attention should be paid to the significant deviations found in the organization of the main aspects of training and after school activities from the generally accepted sanitary and hygienic standards. First of all, this regards the substantial excess of the maximum permissible duration of the daytime stay of students in the training as well as the length of time that was required for practical training and homework outside the educational institution.

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Work is executed within the framework of doctoral dissertation «Scientific bases of complex hygienical estimation of quality of life and adaptation possibilities of student's and student young people that is in the conditions of modern establishments of formation of different types»

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Conflict of interest:

The Authors declare no conflict of interest.

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REVIEW ARTICLE

IMPACT OF PANDEMIC COVID-19 ON THE LEGAL REGULATION OF WORLD TRADE ACTIVITY USING THE EXAMPLE OF THE MEDICAL SUPPLIES

DOI: 10.36740/WLek202007139

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ABSTRACT

The aim of the article is a theoretical and methodological justification for strengthening the role of international trade in medicines and equipment during the COVID-19 pandemic, to establish the benefits of free trade, to develop important international trade policy priorities in this field.

Materials and methods: Legislation of foreign countries and international organizations, statistical data published by international organizations are the materials of the research. Methods of analysis, synthesis, generalization were applied during the research.

Conclusions: It has been proved that the legal regulation of world trade activity by medical supplies are increasingly playing more important roles in the implementation of international development goals. The authors have offered three priorities to all countries: to cooperate globally around the deployment of life-saving medical supplies, publicly commit all countries not to implement export bans or limits on relevant medical supplies; to control all trade measures countries have taken in response to the COVID-19 outbreak; to bring together in a global form measures and procedures to eliminate this phenomenon.

KEY WORDS: global health, public health, international law, international trade, international relations, international organizations, medical supplies, devices and equipment, trade policy

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INTRODUCTION

One of the goals of the UN Sustainable Development for 2016-2030 is to ensure a healthy life and promote well-being for all ages. On March 11 The World Health Organization declared COVID-19 a pandemic. The COVID-19 outbreak is an international concern in the public health. The virus, that first was detected in China on December 2019, has now been detected in nearly 90 locations internationally and has been a significant impact on every aspect of lives countries.

The threat to global health has reached alarming proportions and has exposed a lack of national preparedness and international solidarity. The current role of achieving sustainable development goals is largely the provision of supplies for international trade in medicines and equipment. International law tends to be responsive, to war, atrocity or other disasters. So international law must tend to be responsive also to Coronavirus outbreak, because global co-operation in relation to health, technology, trade and investment is inevitable. Moreover, in an economically interdependent world, the importance of an improved legal framework for promoting international trade and investment is widely recognized.

THE AIM

The aim of the article is a theoretical and methodological justification for strengthening the role of international

trade in medicines and equipment during the pandemic COVID-19, to establish the benefits of free trade, to develop important international trade policy priorities in this field.

MATERIALS AND METHODS

This study was conducted during January-April 2020. The main materials of the research are the norms of the WTO system of agreements, the decisions of 54 countries, that adopted temporary export restrictions on certain categories of critical medical supplies in response to COVID-19. Also this study is based on the empirical and analytical data of the WHO, WTO, the Organization for Economic Co-operation and Development, European Commission. The empirical and statistical data collected were processed, summarized, and analyzed using descriptive statistics capabilities. Totally 85 laws and papers were analyzed.

The key methods used in this research are data analysis, summarization and comparison. The data synthesis and analysis are the key value-added elements of this research, which could help to find out the role of international trade in medicines and equipment during the pandemic COVID-19. The method of system analysis made it possible to formulate the role and the priorities of international trade by medical supplies, devices and equipment during the pandemic COVID-19. The formal and legal method

was used in the analysis of WTO system of agreement and legislative acts of different countries that adopted temporary export restrictions on certain categories of critical medical supplies in response to COVID-19. The statistical method, logical methods, and comparative analysis method were used to analyze the data from the WHO, WTO, the Organization for Economic Co-operation and Development, European Commission, as well as own findings.

REVIEW AND DISCUSSIONS

In today's world, one of the main drivers of economic development is international trade, which is the sphere of international economic relations and represents the sum total of foreign trade in goods; services, products of intellectual labour of all countries of the world. Today, international trade accounts for 80% of all international relations. In particular, international economic cooperation is one of the main factors influencing the level of global progress and economic development of each country today. Each country of the world conducts its own policy, creates its own economy, enters into a wide variety of international relations, carries out international trade, international transportation. Moreover, thanks to intensified trade ties, international relations have become stronger and world peace has move forwarded.

For the first time, world economies are facing an economic and financial crisis, caused not by distortions of market mechanisms, but by the direct consequence of a slowdown in health-related economic growth. The COVID-19 pandemic represents an unprecedented disruption to the global economy and world trade, poses a serious risk to the macroeconomics by halting production activities and shutting down supply networks. Plenty of countries around the world have either not paid sufficient attention to pandemic planning, having apparently presumed that such a scenario would ultimately become China's problem. However, COVID-19 knows no borders. Whether these borders are international frontiers, disciplinary boundaries, or industry sectors, it is clear that modern world need to work together to understand the wide-ranging implications of COVID-19. The dynamics of the pandemic are very different than other types of crises. The COVID-19 outbreak continues to grow rapidly around the world. The disease has been reported in 180 countries [1]. The appearance of a new infectious disease is always a complex situation, especially if it is an epidemic of significant extension or severity [2].

Today virtually all countries are finding themselves with decisions to make as they attempt to deal with the COVID-19 outbreak. The COVID-19 crisis has laid bare stark weaknesses in the health care systems, from the number of intensive-care beds to the size of the workforce, the inability to provide enough masks and to deploy testing in some countries, and deficiencies in the research for and supply of drugs and vaccines [3]. The COVID-19 pandemic has brought considerable attention to trade in medical products and supplies, and specifically trade in products for prevention, testing and treatment. Medical products, in general, are widely spread in different Chapters of the

Harmonized System (HS) classification. A set of products that are considered relevant to COVID-19 prevention and medical treatment in general are categorized into four main groups: 1. Medicines (Pharmaceuticals) – including both dosified and bulk medicines; 2. Medical supplies – refers to consumables for hospital and laboratory use (e.g. alcohol, syringes, gauze, reagents, etc); 3. Medical equipment and technology; 4. Personal protective products – hand soap and sanitizer, face masks, protective spectacles. The protective garments for surgical/medical use are not included in the analysis, because it is impossible to distinguish them from general clothing product in the HS classification.

A subset of medical products has been frequently mentioned by governments, international organizations and in news reports as in short supply for the fight against COVID-19. These include: disinfectants/sterilization products; face masks; gloves; hand soap and sanitizer; patient monitors and pulse oximeters; protective spectacles and visors; sterilizers; syringes; thermometers; ultrasonic scanning apparatus; ventilators, oxygen masks; X-ray equipment; and other devices such as computer tomography apparatus [4]. World experience shows that there is a shortage of essentials such as face masks, test kits, personal protective equipment, fans, ventilators and other items needed by health and safety personnel, has reached crisis levels worldwide.

Medical supplies, devices and equipment play an important role in helping doctors analyze and monitor patients. They can also save the life of thousand people. For the immediate response to the current wave of the pandemic, policy should focus on the availability of sufficient diagnostic tests and emergency supplies. This can be done through international cooperation in purchasing, to avoid excessive purchases and stockpiling in one place creating shortages in others. It also requires demand planning, monitoring supply chains and ensuring that sufficient funds are allocated procuring basic goods that are simple to produce but for which margins and manufacturing capacity may be low [5, 9].

The World Health Organization (*hereinafter referred to as WHO*) has warned that Shortage of personal protective equipment endangering health workers worldwide, and severe and mounting disruption to the global supply of personal protective equipment is putting lives at risk from the new coronavirus and other infectious diseases [6].

In the light of the above, international trade is gaining importance role in ensuring the availability and affordability of vital medicines, medical products and health care services, particularly among its most vulnerable members. International trade represents the total amount of exports and imports of a country and constitutes the most important component of the balance of payments [7]. Trade policies are powerful drivers of the distribution of power, money, and resources, which affect people's daily living and working conditions, their health-related preferences and behaviors, and ultimately their health outcomes [8].

It is against this background that international trade in medical devices and equipment are increasingly playing more important roles in the implementation of international development goals. The priority for governments and the

global community is to prevent people from contracting the disease and to cure those who do. Many countries source these medical supplies from abroad and so trade policy stance becomes part of national policy responses to the Coronavirus.

Increased trade and trade liberalization is a defining feature of globalization, directly and indirectly affecting health and health systems. Diffusion of health technologies through global trade has contributed greatly to worldwide health improvements [9]. Trade policies are powerful drivers of the distribution of power, money, and resources, which affect people's daily living and working conditions, their health-related preferences and behaviors, and ultimately their health outcomes [8].

Today international trade is crucial to ensuring access to medicines and other medical products. The global medical devices market offers tremendous opportunity for manufacturers, as well as significant challenges, for government policymakers seeking to support export competitiveness in overseas markets. *In spite of the fact that* trade in these products collectively amounts to 1.7% of world merchandise trade, world exports of medical products grew by 9% in 2018 and 6% in 2019, from \$859 billion in 2017 to around \$995.8 billion total world exports in 2019 [10]. The United States, the European Union (*hereinafter referred to as EU*), Japan, and China together account for about 90 percent of global production and consumption of medical devices [11]. China is the top exporter of face masks with 25% share. China supplied 25% world exports of face masks in 2019, and together with Germany and the US, the three contribute to almost half of the world face mask supply. Singapore, US, Netherlands, and China export more than half the world's respirators and ventilators. Breathing apparatus, including respirators and ventilators, are supplied by a small number of Members notably, Singapore which has 18% market share, followed by the US with 16%, Netherlands 10% and China 10% [10]. For example, the main destination for EU exports of medicinal and pharmaceutical products in 2019 was the United States (accounting for 32% of EU exports of these products), followed by Switzerland (11%), the United Kingdom (10%), China (6%), Russia and Japan (both 4%). Germany was the largest exporter of medicinal and pharmaceutical products (€47 billion), followed by Ireland (€32 billion), Belgium (€28 billion), the Netherlands (€22 billion), France (€17 billion) and Italy (€16 billion) [12].

The immediate response for COVID-19 has been to break interconnections as a measure to contain and mitigate the virus. This reduces the ability for countries to cooperate, and creates further incentives for countries to implement export bans and anticipate global shortages. These bans on medical supplies have become as infectious as the virus itself. Regrettably we can observe how governments worldwide have started to introduce controls on certain trade activities, particularly relating to the export of medical products and protective equipment, in response to the virus outbreak. Since the beginning of 2020 the governments of some nations have taken steps to ban or limit the export of medical equipment (such as masks) and medicines and their ingredients.

According to the *Global Trade Alert* 54 governments had introduced export curbs on key medical supplies since the beginning of the year [13]. Countries restricting pandemic-related exports include Albania, Argentina, Australia, Brazil, Colombia, India, Kazakhstan, Pakistan, Paraguay, South Korea, Thailand, Vietnam [14]. Of the 164 World Trade Organization (*hereinafter referred to as WTO*) members, only 50 do not tax imported medical devices and 23 levy duties at less than 3% of shipment value. Sensibly, 76 nations do not tax imported medicines. But only 37 WTO members refrain from taxing imports of disinfectant. For soap just nine WTO members permit duty-free imports [15, 1]. The EU has banned for member states the export of personal protective equipment, including face shields, surgical masks and gowns, without export authorization. Regarding trade with non-EU countries, the European Commission issued an Implementing Regulation requiring that exports of specified personal protective equipment, whether or not originating in the European Union, to non-EU countries, be subject to authorization. As specified in a newer Guidance note, this includes all non-EU countries, including the United States and the EU's preferential trade partners, with the exception of the four member states of the European Free Trade Association (Iceland, Liechtenstein, Norway, and Switzerland), certain overseas countries and territories listed in Annex II of the Treaty on EU, as well as the Faeroe Islands, Andorra, San Marino, and Vatican City. The United Kingdom is considered to be an EU country in this context. Exports of certain quantities of specific products may be authorized under specific circumstances such as to ensure assistance to third countries and depending on the needs of the Member States [16].

In today's world, what one country did can have an impact on others. The primary aim of those protectionist policies is protect the provision of public health [7]. However, cutting exports to many countries is risky for two reasons. First, it endangers countries that are also suffering from COVID-19, especially those that rely heavily on supplies. Second, it could endanger the country itself if foreign partners are unable or unwilling to supply with the medical products it needs and cannot produce. Retaliation by foreign partners could even lead to the breakdown of regional and global value chains for essential products. They contribute to the deterioration of the international trade climate at a time when international cooperation is most needed because of the global pandemic. For example, the 25 nations that export significant amounts of medical ventilators include one just nation in Latin America and no nations in Africa, the CIS region, the Middle East, and South Asia. Given the sophisticated technology found in cutting-edge ventilators, it is unlikely that there are local producers in these countries capable of meeting global standards. Therefore, billions of people in developing countries are dependent on international trade for access to this critical technology, used to help patients suffering from advanced stages of COVID-19 [17].

Moreover, as developing countries have a considerable effect on the world market, their decisions to implement

policies to backing their exports or substitute imports will have an impact on all over the world. It should be mentioned here that protectionist policies to international trade have impacts on both an implementing country and other trading countries in a globalized economy. Indeed, the country that implements protectionist policies can influence the world trade according to its size. If that country is a small scale one, the implemented tariffs increase domestic prices while not affecting the world's supply-demand balance. On the other hand, large scale economies have a huge impact on the world economy, and thereby, influence other countries adversely and lead to welfare loss when they employ protectionist policies.

Experience shows that no country can solve the world's problems alone, no country is entirely self-reliant for the products and equipment it needs for its public health systems. In this context, it is naturally worrying that developments have led to a situation in which many countries have difficulty with personal protective equipment, face masks, fans and other items that help to combat coronavirus. For our opinion, regrettably emergency actions must not impose unwarranted restrictions on trade. The government response to the global *crisis* has further heightened economic inequalities an important question which arises in this connection is the effect of the agreements WTO.

According the Resolution of the United Nations General Assembly "70/1 Transforming our World: the 2030 Agenda for Sustainable Development" international trade is a mechanism for the implementation and activation of global partnerships. *A universal, rules-based, non-discriminatory and equitable multilateral trading system is the best modality to advance trade among member States and can promote the economic growth and advancement of developing countries. Through transparent, non-discriminatory and fair rules for trade regulation, the promotion of exports by developing countries, duty-free and quota-free access to the markets of the least developed countries, trade should promote inclusive development, environmental sustainability and economic growth* [18].

Today, the regulation of international trade is carried out through the WTO system of agreements. The WTO acknowledges that health is a legitimate policy goal and provides an exception rule that is contained in most trade agreements, which allows member states to introduce health-related policy measures provided they are deemed necessary to protect human or environmental health and safety and are not introduced to act as a barrier to trade [8]. During the 20 years of its existence, the WTO has transformed trade into a tool for socio-economic growth, sustainable development, environmental protection, fostering innovation and creating fair market conditions for business. The complexity of relationships and business structures, the change of traditional paradigms of world trade, economic crises in this situation of spread Covid-19 pandemic significantly complicate the functioning of the organization, forcing the latter to respond in time to change.

The WTO Rules permit Members to take actions to protect the health and safety of their citizens, in particular in particular

in the General Agreement on Tariffs and Trade (GATT 1947) [19], the General Agreement on Trade in Services [20], the Agreement on Trade-Related Aspects of Intellectual Property Rights [21], the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) [22], the Agreements on technical barriers to trade (TBT Agreement) [23].

One of the key rules affecting exports is Article XI of the General Agreement on Tariffs and Trade (GATT 1947): on General Elimination of Quantitative Restrictions which specifies that 'no prohibitions or restrictions other than duties, taxes or other charges, whether made effective through quotas, import or export licenses or other measures, shall be instituted or maintained by any contracting party on the importation of any product of the territory of any other contracting party or on the exportation or sale for export of any product destined for the territory of any other contracting party'. Certain exceptions to this general prohibition are allowed under Article XI: of the GATT. In particular, temporary prohibitions or restrictions were allowed to relieve critical shortages of foodstuffs or other products essential to the exporting contracting party. Article XX contains an exception from these commitments for actions taken that are "necessary to protect human... life or health," subject to the proviso that "such measures are not applied in a manner which would constitute a means of arbitrary or unjustifiable discrimination between countries where the same conditions prevail, or a disguised restriction on international trade..." [19; 24].

The Agreement on Trade-Related Aspects of Intellectual Property Rights also provides members with flexibilities to ensure that life-saving drugs are available and affordable for their citizens. Among other tools, governments can use WTO-compliant compulsory licensing procedures in cases where patented drugs have been unaffordable or not widely available [21]. The Agreement on the Application of Sanitary and Phytosanitary Measures establishes that members have the right to restrict trade by taking measures necessary for the protection of human, animal or plant life or health. These measures should only be applied to the extent necessary to achieve their objectives, be based on scientific principles and be supported by scientific evidence. In situations where relevant scientific evidence is insufficient, members may provisionally adopt measures on the basis of available pertinent information [22]. The Agreements on technical barriers to trade aims to ensure that technical regulations, standards and conformity assessment procedures are non-discriminatory and do not create unnecessary obstacles to trade. At the same time, it recognizes WTO members' right to implement measures to achieve legitimate policy objectives, such as the protection of human health and safety [23].

All these agreements require WTO members to notify others of any new or changed requirements which affect trade, and to respond to requests for information on new or existing measures. *In addition*, these measures should be applied in such a way that there is no discrimination between WTO members and should not be a disguised restriction on international trade.

The WTO rules provide a wide scope for Member States for trade relations. At the same time, membership in the WTO imposes on the Member State specific obligations, which in content can be fairly conditionally grouped into the following groups: first, these are changes affecting the national trade regime as a whole (import and export duties, rules and procedures for customs valuation and origin of goods, principles for applying safeguards in trade, etc.); secondly, the introduction at national level of international principles and procedures for the application of technical norms and standards, sanitary and phytosanitary measures, etc., i.e. tools for ensuring consumer safety; thirdly, it is a change of the national regime of regulation of certain sectors of industry and agriculture, service sector.

An analysis of the WTO agreements makes it possible to conclude that these agreements nothing in the WHO rules prevents a roll-back of export restrictions, nothing subsidies from being granted to increase the global supply of medical products needed to respond to the COVID-19 pandemic. Also additional flexibility in the WTO's Agreement on Trade Related Intellectual Property can facilitate countries' access to affordable life-saving medicines.

Along with this there are several other important issues: whether tariffs can be eliminated on medical supplies, whether export restrictions on these products can be lifted if already imposed, and eliminated if already in place, and whether subsidies are needed to spur necessary production and economic activity? Progress in answering these questions has been notably slow. If there is to be a rule of thumb to follow in answering these questions, it should be to remind all developed countries that the COVID-19 pandemic is a challenge for societies around the world, which means that we all have a shared responsibility for this together. The human costs of the Coronavirus pandemic are already immeasurable and all countries need to work together to protect people and limit the economic damage.

It is too early to gauge the full economic and trade effects of the Coronavirus outbreak. There will undoubtedly be many unanticipated consequences of this viral outbreak, economically, politically and from a global health response perspective. The domestic policies need to be supported by maintaining international trade and cooperation, which are essential to defeating the pandemic and maximizing the chances of a quick recovery. Bearing in mind that the reactivation of economic growth and sustainable development in all countries requires, *inter alia*, a dynamic and a supportive international economic environment, three important priorities should be identified.

The first priorities have sensibly been to combatting this pandemic calls for a transparent, robust, coordinated, large-scale and science-based global response in the spirit of solidarity [25]. The COVID-19 pandemic has highlighted the need for greater cooperation and efforts to reduce barriers to trade, including through increased mutual recognition agreements. These are the actions that leaders should be taking now. Instead of restricting the international trade by medical supplies, devices and equipment, it is necessary, on the contrary, to cooperate globally around the

deployment of life-saving medical supplies, publicly commit all countries not to implement export bans or limits on relevant medical supplies or reverse existing export bans on medical supplies needed to tackle the Coronavirus. The international system for regulating international trade by medical supplies, devices and equipment must address the needs of the rich and the poor. When patented medicines are not affordable, governments must act [26]. The coronavirus crisis has highlighted the challenge of protecting the health of the population whilst avoiding disruptions to the free movement of persons, and the delivery of goods. The next natural progression is for the governments to cooperate with the medical industry to boost production and set a ceiling price for these items. Governments and industry will need to come to an agreement: during a pandemic, vaccines and antivirals can't simply be sold to the highest bidder. They should be available and affordable for people who are at the heart of the outbreak and in greatest need. Not only is such distribution the right thing to do, it's also the right strategy for short-circuiting transmission and preventing future pandemics [27]. Governments should develop incentives for industry to ramp up production. This includes easing restrictions on the export and distribution of personal protective equipment and other medical supplies. In this way we can observe how the European Commission has sought to help the industry address the urgent need for health care during the pandemic. In the Commission Recommendation (EU) 2020/403 of 13 March 2020 on conformity assessment and market surveillance procedures within the context of the COVID-19 threat The European Commission asked the European Committee for Standardization (CEN) and the European Committee for Electrotechnical Standardization (CENELEC) to provide a number of European standards for certain medicines and personal protective equipment [28].

The second priority is to control all trade measures countries have taken in response to the COVID-19 outbreak. For the realization this goal should govern the conduct of policies towards medical supplies the COVID-19: international trade by medical supplies, devices and equipment should be transparency and coherence, do not harm. Notwithstanding all the challenges, the WTO will remain an effective regulator of international trade at the multi-lateral level, and the authorities, business and citizens will increasingly feel the beneficial effects of international trade and the socio-economic development of states.

Nowadays the WTO, the United Nations Department for Economic and Social Affairs and the International Trade Centre have been created the web page "ePing" for allowing users to receive daily or weekly email alerts about new sanitary and phytosanitary (SPS) information or technical barriers to trade (TBT) information notifications and to search for existing measures covering products or markets of interest to them. WTO Members are normally required to inform each other when planning to change product requirements and provide an opportunity for other Members to comment on these requirements. They do so by submitting a two-page info sheet on the regulation with

information on products covered, a brief summary of the regulation and the deadline for providing comments. These info sheets, called SPS or TBT notifications, are sent to ePing users based on their interests. Today there are more than 63000 notifications [29].

The third priority is to bring together in a global form measures and procedures to eliminate this phenomenon. International cooperation is essential to deal with the health and economic impact of the COVID-19 virus. Global cooperation is the only way countries can minimize the disruption that COVID-19 leaves in its wake. International cooperation is critical to ensure medical supplies ultimately reach the most vulnerable populations. Close coordination between action by states and action by the international community is a key element of this crisis, in particular to ensure that essential commodities needed to mitigate health risks from outbreaks can reach anyone who needs them. It is important to develop policy mechanisms to improve common approaches to ensure the production, storage, accessibility and rational use of medicines, and not to take unilateral measures that restrict the free movement of essential medical supplies.

CONCLUSIONS

While the COVID-19 pandemic is first and foremost a health crisis, its implications are more far-reaching and could threaten global peace and security. Overall, a global trade cooperation is a crucial and needed solution to fight COVID-19. The human costs of the Coronavirus pandemic are already immeasurable and all countries need to work together to protect people and limit the economic damage. In the global cooperation all countries should commit to take all necessary health measures and seek to ensure adequate financing to contain the pandemic and protect people, to continue working together to facilitate international trade and coordinate responses in ways that avoid unnecessary interference with international traffic and trade. All countries need to work to ensure the flow of vital medical supplies, and work to resolve disruptions to the global supply chains, to support the health and wellbeing of all people. Sharing information and facilitating the flow/movement of medical products can be one of many forms of global cooperation in sharing global resources to combat this pandemic. It should, in particular, seek to promote effective implementation of the existing commitments and agreements in the area of international economic and social development.

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REVIEW ARTICLE

CLINICAL AND SEROLOGICAL PARAMETERS OF PROGRESSION AND PROGNOSIS IN PATIENTS WITH SYSTEMIC SCLEROSIS – A STATE OF THE ART REVIEW

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ABSTRACT

Systemic sclerosis (SSc) is a multi-organ connective tissue disease that leads to the dysfunction and the impaired morphology of blood vessels due to non-specific inflammation and progressive fibrosis. Due to the diversity of SSc and even though the factors predisposing to the severe course of SSc are known, it is not always possible to predict the disease progression and to determine the prognosis. Ideally, the group of patients with faster progression of organ lesions and a worse course of the disease should be identified and the early intensive treatment should be instituted.

The aim of the article, is an attempt to identify the factors that worsen the prognosis in the course of SSc.

The analysis of numerous studies demonstrated that patients with short-lasting SSc, with the presence of anti-RNA polymerase III antibodies, with a generalized type of SSc with quickly progressing skin lesions and males should be most strictly monitored. Moreover, vascular complications, tendon ruptures and fast capillaries loss observed in nailfold capillaroscopy are the factors deteriorating the prognosis in SSc.

In conclusion, despite the known, the factors that worsen the prognosis, it is difficult to predict the course of systemic sclerosis. Due to its incompletely elucidated etiopathology as well as the diverse and unpredictable nature of the disease, reliable markers to determine the prognosis in SSc have not been found.

KEY WORDS: systemic sclerosis, a course of the disease, poor prognostic factors

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INTRODUCTION

Systemic sclerosis (SSc) is a multi-organ disease that belongs to systemic connective tissue diseases. The etiopathogenesis of the disease is not fully understood, although in early stages endothelial damage occurs with the disturbance in the morphology and the function of small blood vessels, inflammatory process and activation of the immune system and fibroblasts. The consequence of these processes is the damage to the vessels with progressive fibrosis of the skin and internal organs, especially the lungs, heart, digestive tract and kidneys. The course of SSc is very diverse, therefore in many cases we are not able to predict the consequences of the disease, and the degree of internal organ involvement as well as to determine the prognosis. The beginning of SSc is also heterogeneous. There is a group of patients with a long-term stable disease from the beginning of its onset, in whom no serious organ symptoms develop. On the other hand, the course can be very progressive. Then in a short time after or at the same time with the appearance of first symptoms of the disease, severe internal organ involvement occurs, even with sudden cardiac death or severe respiratory failure. Canadian physician William Osler who lived in 1849-1919 called SSc “one of the most terrible of all human ills” [1].

Adverse prognostic factors in systemic sclerosis

Despite the known factors indicating a more severe SSc course, we are not always able to predict the progression of the disease and to determine the prognosis [2]. Based on the literature data [2, 3], the known factors of the poor prognosis of SSc include:

- generalized type of the disease,
- early involvement of internal organs, especially lungs, heart or kidneys,
- high inflammatory parameters
- male gender.

On the other hand, a milder course of the disease can be demonstrated by:

- limited type of SSc,
- absence or benign involvement of internal organs,
- presence of anti-centromere antibodies
- overlap SSc with other autoimmune diseases.

The optimal strategy would be to identify a group of patients who will have a faster progression of organ changes and a worse course of the disease, and to implement early intensive treatment in this group (Table 1)).

THE AIM

The aim of the article, is an attempt to identify the factors that worsen the prognosis in the course of SSc.

Table 1. Analysis of the significance of selected prognostic factors in systemic sclerosis

Prognostic factor	Worse prognosis	Better prognosis
Demographics	Male sex	Female sex
SSc type	Generalized	Limited
Presence of antibodies	Anti-RNA polymerase III antibodies	Anticentromere antibodies
Inflammation parameters	High ESR or CRP	Normal ESR and CRP
Skin involvement	Fast skin lesions progression	Slow skin lesion progression
Internal organs involvement	Early involvement of lungs, heart and kidneys or tendons rupture	No or mild involvement of internal organs
Lung function tests	FVC < 70%, fast decrease in DLCO	FVC > 70% DLCO > 80%
High resolution computed tomography	Pulmonary lesions extension > 20%	Pulmonary lesions extension < 10%
Vascular complications – fingertips ulcers	Fingertip ulcerations	No fingertip ulcerations
Capillaroscopy	Fast capillaries loss	No or slow capillaries loss

FVC – forced vital capacity, DLCO – [%] predicted diffusing capacity for carbon monoxide, CRP – C-reactive protein, ESR – erythrocyte sedimentation rate

REVIEW AND DISCUSSION

Is it possible to predict the course of the disease based on the assessment of skin lesions?

Harrick et al. in their research evaluated patients with early generalized SSc with rapid progression of skin lesions and attempted to predict the course of the disease during 24 months of follow-up [3]. In a group of 326 patients with SSc, the degree of skin involvement was assessed every 3 months. The extent of skin lesions was determined by means of skin fold thickness assessment, defined as Rodnan skin score (RSS) [4]. The skin fold thickness was assessed by palpation on a scale of 0 to 3 points in 17 different areas of the body. An increase by 25% and more than 5 points in RSS was considered to be a progression risk factor. The authors of the study showed that during 24 months of follow-up RSS increased by 25% in 22.5% of patients. This group of patients characterized a shorter duration of the disease and a lower RSS at the beginning of the study [3]. The study also analyzed a group with the presence of anti-RNA polymerase III antibodies and found that more than 29% of patients with these antibodies had a 25% increase in RSS over the 2 years of follow-up. It was also shown that this group with the presence of anti-RNA polymerase III antibodies had the highest RSS at the start of the study, the highest maximum rate of skin involvement during follow-up compared to the group of patients with SSc without the presence of anti-RNA polymerase III antibodies. Summing up the study, authors showed that the group of patients with SSc who baseline had a lower RSS and a shorter duration of the disease presented a greater progression of skin lesions during the 24 months of follow-up. This indicates that the baseline value of RSS is not a sufficient prognostic risk factor. In addition, patients with a short duration of the disease and with the presence of anti-RNA polymerase III antibodies should be closely monitored [3].

MORTALITY IN SYSTEMIC SCLEROSIS

Hao et al. examined a group of 1,070 patients with SSc lasting less than 4 years and compared with a group of

3,218 patients with SSc lasting longer than 4 years [2]. The mortality rate, life expectancy and age at death were assessed in both groups. It was shown that the mortality rate was higher in the group of patients with SSc with a shorter duration of the disease, and especially in men with a shorter duration of the disease. It was also shown that the mean life expectancy in the group of patients with a shorter duration of the disease was lower. In addition, in the group of SSc patients with a duration of less than 4 years, there were 140 deaths, of which over 60% were deaths associated with SSc, and interstitial lung disease or pulmonary arterial hypertension were the main causes. Cancer, sepsis, cerebrovascular disease and ischemic heart disease dominated among causes of non-SSc deaths. Analyzing a group of patients with a longer duration of the disease, 440 deaths were recorded, of which over 50% were associated with SSc. It was shown that risk factors for death in the group of patients who had the disease for less than 4 years were male sex, more advanced age at the time of diagnosis, generalized SSc, pulmonary arterial hypertension and scleroderma renal crisis, while a longer duration of the disease was the protective factor indicating a better prognosis in this group. In the population of patients with SSc, whose disease duration exceeded 4 years, the risk factors for death were also male sex, more advanced age at the time of diagnosis, generalized SSc, pulmonary arterial hypertension and, in addition, interstitial lung disease, while longer duration of the disease and the presence of anti-centromere antibodies were factors associated with better prognosis. To sum up this research, early mortality in SSc is significant, especially in the group of patients with the duration of the disease is less than 4 years, and especially in males and in case of generalized SSc [2, 5].

Can the prognosis of interstitial lung disease in the course of systemic sclerosis be predicted?

Predicting the course of SSc in patients with interstitial lung disease is very challenging. Already in 2008 Goh et al. demonstrated the relationship between the extent of lung lesions assessed in high resolution computed tomography

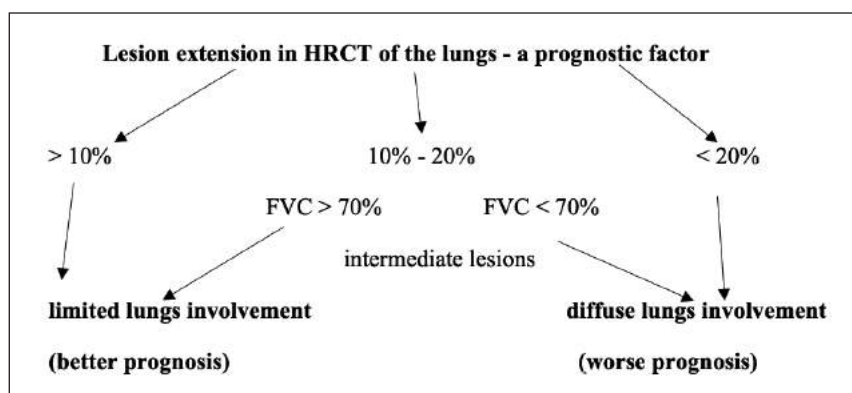


Fig. 1. Prognosis in interstitial lung disease in systemic sclerosis based on the assessment of the extent of pulmonary lesions in HRCT [6]. HRCT – high resolution computed tomography

(HRCT) and the prognosis in interstitial lung disease associated with SSc [6]. Authors revealed that in case of pulmonary lesions below 10% in lung HRCT, there is limited lung involvement and a better prognosis. In case of lung involvement above 20% with diffuse lung involvement authors disclosed a worse prognosis. Pulmonary changes between 10% – 20% in lung HRCT are considered intermediate and then pulmonary function tests are important. In case of intermediate pulmonary changes, if forced vital capacity (FVC) is above 70%, then the prognosis is better, and FVC below 70% is associated with a worse prognosis (Fig. 1) [6].

In 2017, Moriset et al. assessed the risk of death during the first 3 years of SSc duration with associated interstitial lung disease based on the SADL model (S – ever smoking history, A – age, DL – DLCO [%] predicted diffusing capacity for carbon monoxide) [7]. The study involved 225 patients with interstitial lung disease in the course of SSc and based on the SADL model, the percentage of patients with a low, medium and high risk of death was determined. 21% of patients had a high score calculated on the basis of the SADL model. The highest risk of death was found in the group of patients with high scores in the SADL model [7]. In addition, smoking, more advanced age and rapid decline of DLCO were shown to be death risk factors in the first years of SSc with interstitial lung disease. To sum up, on the basis of the SADL model it is possible to distinguish a group of patients with SSc with interstitial lung disease and with a high risk of death during the first 3 years of the disease, and to some extent to predict the prognosis in this group of patients [7]. In another research, Wu et al. showed that a decrease in saturation below 94% during a 6-minute walk test and a history of arthritis were independent risk factors for the progression of interstitial lung disease in the course of SSc during one-year follow-up [8]. In 2019, a large multicenter study of the EUSTAR group – European Scleroderma Trials and Research Study Group – covering over 1,000 patients was published. It revealed that the progression of skin lesions within one year were associated with worsening of lung function tests and worse survival in patients with generalized SSc [9, 10].

Relationship between vascular changes and prognosis in systemic sclerosis

Many studies were conducted to determine the relationship between vascular changes and joint symptoms

in predicting the course of the disease and assessing the prognosis in patients with SSc. One of the large multicenter studies conducted by the EUSTAR group evaluated over 3,000 patients with SSc for the presence of ulcers on fingertips. Fingertip ulcerations (present or in history) were found in 34% of patients. Authors demonstrated that the history of fingertip ulcers was a predictive factor for the development of new ulcers, cardiovascular complications, including pulmonary arterial hypertension and shortened life expectancy [11]. Another study of the EUSTAR group focused on the association of musculoskeletal symptoms with the prediction of SSc progression. Arthritis and tendon rupture were found to be independent factors in the progression of skin lesions in the course of SSc. In addition, arthritis was a predictor of new fingertip ulcers and worsening of left ventricular ejection fraction. In addition, tendon ruptures were shown to be independent risk factors for the development of scleroderma renal crisis. In conclusion, arthritis and tendon rupture were independent predictors of the disease progression in the early phase of SSc [12]. A number of studies was also carried out to assess whether, based on abnormal nail-fold capillaries on capillaroscopy examination, it was possible to predict the degree of internal organ involvement and determine the prognosis. Smith et al. showed that capillaroscopic abnormalities of “late” scleroderma microangiopathy were associated with more frequent future development of vascular lesions and pulmonary complications during 2-year follow-up [13]. Authors showed that 63% of patients with SSc who at the time of enrollment had the image of “late” scleroderma microangiopathy with a significant reduction in the number of capillaries developed peripheral vascular complications, and as much as 73% of pulmonary complications within 18-24 months of follow-up [13, 14]. On the basis of a meta-analysis carried out by Paxton et al., a relationship was found between capillary loss in capillaroscopy examination of nail-fold capillaries and organ complications, in particular vascular disorders such as finger ulcers, development of pulmonary arterial hypertension, as well as the occurrence of calcinosis [15]. In summary, based on the progression of capillaroscopic changes, one can also predict the degree of internal organ involvement and determine the prognosis in SSc. In addition, it was also shown that some biomarkers such as: growth differentiation factor-15

(GDF-15), placenta growth factor (PGF), endostatins, and vascular-endothelial growth factor (VEGF) present in high serum concentrations in patients with SSc are factors increasing the risk of developing a generalized SSc subtype, interstitial lung disease, pulmonary arterial hypertension or fingertip ulceration [16].

Treatment of systemic sclerosis depending on the course and phase of the disease

Identifying adverse prognostic factors in the course of SSc is of particular importance in therapeutic management. It still remains a dilemma whether to intensively treat with immunosuppressive drugs patients with very early SSc, without involvement of internal organs. At a very early stage of the disease, close observation and monitoring of patients with early involvement of internal organs is suggested to identify those in the window of opportunity of therapeutic options and to treat them aggressively so as not to lead to irreversible changes in the course of the disease. One prospective study enrolled 326 patients with an early SSc phase from 50 centers and patients were followed up for 2 years. Patients were divided into four groups. The first group was treated with methotrexate 20-25 mg/week p.o., the second group – with mycophenolate mofetil 2 x 1 g / day p.o., the third group – with cyclophosphamide 500 mg/m² 1 x month i.v. for 6-12 months or p.o. 1-2 mg/kg/day p.o. Methotrexate, mycophenolate mofetil or azathioprine were used as a continuation of the therapy. The fourth group was not treated with immunosuppressive drugs. In the study, no statistically significant differences were found between the four groups, however, not statistically significant improvement was shown in patients treated with immunosuppression. In addition, RSS improved in all four groups, however the smallest improvement was obtained in the group of patients not treated with immunosuppression. In that group there was also a higher mortality rate [17].

CONCLUSIONS

In summary, internal organ involvement in SSc often occurs early in the disease and may be associated with increased mortality. Factors that worsen the prognosis in the course of the disease include:

- male sex,
- a shorter duration of the disease,
- generalized SSc,
- the presence of organ complications such as pulmonary arterial hypertension, interstitial lung disease, fingertip ulceration or tendon rupture.

Factors that improve prognosis in systemic sclerosis include:

- a longer duration of the disease,
- limited SSc,
- presence of anti-centromere antibodies.

The initial high RSS is not always a sufficient prognostic factor for a worse course of the disease, however, rapid progression of skin lesions is still associated with the risk of developing interstitial lung disease. Risk factors for death in interstitial lung disease during the first years of SSc are history of smoking, more advanced age and rapid decrease in DLCO. Changes in the capillaroscopy examination of

the nailfold, especially early capillary loss, correlate with the progression of organ changes, in particular vascular complications and the development of interstitial lung disease in SSc. Despite the generally known factors of poor prognosis, in many cases it is difficult to predict the course of SSc and we are still not able to isolate sufficient markers that would allow an unambiguous assessment of the prognosis.

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Conflict of interest

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REVIEW ARTICLE

MEDICAL AND PROCEDURAL-LEGAL ASPECTS OF INPATIENT AND OUTPATIENT FORENSIC PSYCHIATRIC EXAMINATION

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ABSTRACT

The aim: The aim of the study is to define the legal framework of forensic psychiatric examination commissioned by the court in relation to the competence of medical practitioners and the position of the subject as a patient in the process of forensic psychiatric examination in order to determine the correlation of special legal regulation with criminal and civil procedure regulation and to make proposals for the enhancement of the legal regulation.

Materials and methods: This study is based on the analysis of international law, medical civil procedure and criminal procedure legislation, juridical practice, medical law legal doctrine. The following methods were used in this research: the method of interpretation of legal norms, analysis of legal acts, and the induction-deduction method, upon which the conclusions were drawn and recommendations were provided.

Conclusion: The current regulatory framework does not provide for the procedure by which the subject's medical treatment is conducted during forensic psychiatric examination, nor does it determine the criteria for the admissibility of treatment of the persons concerned and the extent of treatment. During the examination, the medical practitioner who is in the expert's procedural position in relation to the subject under examination in the particular examination should not carry out the treatment of the subject.

KEY WORDS: accountability, legal capacity, criminal proceedings, civil proceedings, medical treatment, expert

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INTRODUCTION

The legal scope of treatment should not be narrowly understood in its generally understood sense of a set of actions aiming to restore human health or prevent disease. Criminal and civil proceedings involve procedural means other than treatment as “professional prevention, diagnosis and treatment of a specific disease, medical rehabilitation and patient care” [1; 2], which are carried out by medical practitioners in criminal and civil proceedings based on the basis of the decision of the person directing the proceedings. It is a forensic psychiatric expert-examination commissioned by the court. Today's mental health experts working in the criminal justice field have an increasing burden of responsibility as a result of their wider role, and potentially greater power to harm [3].

When making the proper forensic qualification of the case and making a lawful and just decision in the case, the forensic psychiatric examination plays an important and sometimes decisive role.

Forensic psychiatric assessment regarding liability ensures the balance between justice and psychiatry [4]. Such an examination is necessary in many cases, but its conduct should not lead to a violation of fundamental human rights and freedoms.

Criminal liability applies to a person, who is an essential principle of criminal procedure, while in civil law, actions conducted by the person in the state of mental incapacity may be invalidated, thereby terminating the existing legal relationship or preventing its establishment.

THE AIM

The aim of this research is to define the legal framework of forensic psychiatric examination in relation to the competence of medical practitioners and the position of the subject as a patient in the process of forensic psychiatric examination in order to establish the correlation of special legal regulation with criminal and civil procedural regulation and make proposals for enhancing the regulatory framework.

MATERIALS AND METHODS

This study is based on the analysis of international law, medical civil procedure and criminal procedure legislation of the Republic of Latvia and Ukraine, the authors' own experience in legal practice, medical law legal doctrine. Totally 14 laws and papers were analyzed. There were used the methods of interpretation of legal norms, analysis of legal acts, and the induction-deduction method, which were followed by conclusions and recommendations.

REVIEW AND DISCUSSION

The issue of the ability of the natural person, the legal entity, to understand the motives of his or her actions or omissions, to conduct his actions consciously, and to be aware of the consequences of such actions or omissions

is central to deciding on a person's legal liability or the validity of the civil transaction entered into by the person. This total set of such elements is referred to as the institute of legal capacity.

There is a distinction between medical and legal aspects of the legal capacity. The former aspect is inextricably linked to a person's cognitive abilities and derives from a person's mental health. It is logical that the assessment of a person's abilities can only be performed by a person with specialist knowledge – a medical practitioner or a person who has medical education and provides medical treatment [1; 2]. On the other hand, the latter, legal, aspect is related to the objectively determined state of health of the person under investigation and the subjective attitude of this person, which is expressed in the understanding of one's actions or omissions and their consequences. Thus, this is how the state of mental health affects a person's ability to act, which in legal science is interpreted as the ability to understand and take responsibility for one's actions [5]. In summary, the person with legal capacity is a person able to act. However, not always do the medical and legal aspects coincide – a person may not be mentally ill and yet, for a specific offense or transaction, this person may be considered legally incapable or incapacitated, or vice versa – a mentally ill person may be recognized as legally capable. Thus, medical and legal sciences fuse in one process. A judicial officer (the person directing the proceedings – the court or judge), based on the opinion of the medical practitioner, assesses the person's legal capacity.

The issue of declaring a person incapable of committing an offense or of entering into a civil transaction cannot be left to the sole discretion of this individual or the person directing the proceedings. The individual is always interested in the outcome of the case and therefore cannot be impartial, yet, neither he/she nor the person directing the proceedings have specialist medical knowledge, the need for which has already been mentioned. It serves as a legal instrument for the forensic psychiatric examination, which seeks to answer the question of whether the person is considered to have legal capacity for the incriminated criminal offense (criminal law) or for entered transaction, when having legal capacity (civil law).

Expert-examination is a total set of procedural acts, therefore, its procedure is determined by the Latvian and Ukrainian procedural laws – Criminal Procedure Law of the Republic of Latvia [6], Criminal Procedure Code of Ukraine [7], Civil Procedure Law of the Republic of Latvia [8], Civil Procedure Code of Ukraine [9]. Considering the fact that the subject of forensic psychiatric examination is a human being, that is, a living being and this examination pertains to human health, it cannot be denied that the scope of medical expertise includes the application of the Medical Treatment Law of the Republic of Latvia [1], Fundamentals of Ukrainian legislation on healthcare [2], etc., and the legal acts that regulate medical treatment.

In addition, as a total set of medical-procedural activities, this examination creates a collision between the involved persons. The examiner is both a medical practitioner and

an expert (i.e., an official with special statutory rights and obligations). The performer of the expertise is both a medical practitioner and an expert (i.e., an official with the rights and obligations under the specific law).

The person who is subject to the examination has a certain procedural status applicable to him or her in the case, but this does not exclude the extension of this status to the patient's rights. Special attention shall be paid to these issues in the case of inpatient forensic psychiatric examination commissioned by court (hereinafter – examination), when the person's freedom, which is one of the basic human rights, is restricted [10].

According to the scientific literature, in the case of examination, deprivation of liberty formally begins when the fact of conducting examination in criminal proceedings, since it is possible to carry it out under compulsion [11]. In criminal proceedings, a person with the procedural status of a suspect or accused person is *a priori* subject to certain limitations because of his or her status. The situation is different in civil proceedings, in which persons are not subject to state coercive measures because the process is between individuals, and yet, examination is still admissible. Part I of Section 121 of the Civil Procedure Law of the Republic of Latvia provides that the examination is to be determined at the request of a party in cases where special scientific knowledge is required to clarify the relevant facts of the case [8]. The same rules are provided for by Articles 103-105 of the Civil Procedure Code of Ukraine [9]. Thus, in civil proceedings, the significance of the facts to be ascertained for the proper resolution of the case is the legitimate basis for conducting the examination. This involves the duty of the court to ascertain the objective truth, as required by Section I of Article 17 of the law "On the Power of the Court" [12]. Consequently, only the facts which are relevant to the proper settlement of the case may be the subject of a request for examination. The facts to be recognized as significant, first and foremost, pertain to the person's legal capacity. The incapacity of a person destroys the force of a transaction, so the transaction entered into by the incapacitated person is void. This follows from Paragraph 1405 of the Civil Code of the Republic of Latvia [13]. On the other hand, when assessing a person's legal capacity, it is necessary to prove the connection between the person's actual capacity and the restriction of legal capacity [14]. This entails the necessity to prove that at the time of the conclusion of the transaction, the actual ability of the person to be aware of and manage his or her activities match the medical criteria of legal capacity.

In turn, in criminal cases, in accordance with Section I of Paragraph 596 one of the Criminal Procedure Law of the Republic of Latvia [6] and Section 1 of Article 509 of the Criminal Procedure Code of Ukraine [7], the person directing the proceedings shall determine a court psychiatric examination for a suspect or accused, if information has been acquired in criminal proceedings regarding the fact that a person ill with mental disturbances committed a criminal offence while in a state of mental incapacity, or has fallen ill following the committing of the criminal offence.

Mens rea is the mental element of an offense, and psychiatric disorders have the potential to influence the competency or capacity to form any particular intention or behaviour that can lead to a crime. Therefore, psychiatrists are frequently asked to evaluate a defendant's mental state at the time of the offense to determine the required mens rea that is related to the crime. [15]

Legal professionals such as judges can provide a legal assessment of the situation, but cannot assess the medical criteria for a person's legal capacity. The medical criteria are evaluated by experts, specifically, by medical practitioners during the examination.

The forensic experts who are independent and law-abiding officials have the right to conduct such examinations. There are two types of forensic experts in Latvia – public forensic experts and private forensic experts [16]. It should be emphasized that private forensic experts do not have the right to conduct inpatient forensic psychiatric examination, but have the right to carry out outpatient forensic psychiatric examination. In contradistinction to Latvia, according to Ukrainian legislation, the forensic psychiatric examination is carried out only by state expert institutions [17].

In the Republic of Latvia, the Cabinet determines the procedure by which forensic psychiatric examinations shall be performed. Furthermore, it can be concluded that both outpatient and inpatient forensic psychiatric examinations are conducted at forensic institutions, psychiatric treatment institutions or outpatient psychiatric facilities [18]. Similar provisions are also contained in the by-law of Ukraine – the Procedure for conducting forensic psychiatric examination established by the Ministry of Health [19]. It is evident that the examination can be carried out not only in an institution which is institutionally separated, for example, a specialized forensic institution can be recognized as such type of institution, but also in a general psychiatric institution or its outpatient facility. It should be highlighted that the above mentioned Latvian Cabinet of Ministers Regulations [18] have been issued in accordance with Section 72 of the Medical Treatment Law [1], as well as Ukrainian Minister of Health Order [19] have been issued in accordance with Article 71 of the Fundamentals of Ukrainian Legislation on Healthcare [2]. It emphasizes the close connection of forensic psychiatric examination with the treatment and consecutively with the patient's rights. Any person who, by voluntary or involuntary admission to a medical institution due to medical manipulation, enjoys the patient's rights, the extent of which may vary depending on the procedural status of the person concerned. This is an expression of the dual nature of a person's legal state, which correlates the patient's rights with the legal status established in criminal or civil proceedings.

Certainly, the rights of the patient are not absolute, but the fact that a person has been granted a certain procedural status in the case does not in itself justify depriving the person of the patient's rights. To what extent can a patient's rights be restricted if a person is admitted to a medical institution for examination?

When performing forensic psychiatric examination, the person is admitted to a general medical institution and the

patient's rights are applied to him or her. While at a medical institution, the person concerned is a patient. There are no exceptions in the Patient's Rights Law consistently with which it can be argued that this law does not apply to persons admitted to a medical institution for psychiatric examination. A doctor – expert is primarily a medical practitioner who simultaneously provides treatment for patients and works as an expert medical practitioner at the same medical institution.

Forensic psychiatric examination is always based on a reasonable doubt about the person's mental state. Therefore, because of age or illness, a person's cognitive abilities are in doubt. In such cases, the person has often been previously treated for mental illness. Psychiatric care is based on a voluntary basis [1; 2]. This is the way in which patients' rights are respected, specifically, according to Paragraph I of Article 6 of the Patient's Rights Law, medical treatment can be conducted only with the informed consent of the patient [20]. It is only in exceptional cases that psychiatric treatment without the consent of the person and forced placement in a psychiatric institution is permissible. These exceptions are specified in Section 68 of the Medical Treatment Law, namely, (1) the person concerned has threatened or threatens, tried or is trying to do personal injuries to him or herself or to another person or has behaved or behaves violently to other persons and a medical practitioner has determined that the patient has a mental health disorder for which the possible consequences may be personal injury to the patient him or herself or another person; 2) the patient has indicated or indicates an inability to care for him or herself or for a person under his or her guardianship and a medical practitioner has determined that the patient has a mental health disorder for which the possible consequences may be unavoidable and serious deterioration of the person's health.

Evidently, the placement of a person in a psychiatric medical institution for inpatient forensic psychiatric examination is not regulated by the Latvian Medical Treatment Law [1] as well as Fundamentals of Ukrainian legislation on healthcare [2], which is considered to be a deficiency of legal regulation, because in matters related to person's health, to matters pertaining to the person as a living creature being admitted to a medical institution, there is a need for the application of a special law, not the procedural law regulating the procedure for carrying out procedural acts. However, the legal framework requires the examined person to give a written consent for conducting examination [18; 19]. At the same time, it should be emphasized that there are no legal consequences and no further action to be carried out by the person directing the proceedings if the examined person refuses to give his or her consent. The legislator has included contradictory requirements in the legal framework, which do not help to eliminate doubts, specifically, if the examined person is or is not endowed with the patient's rights and in which cases the forensic psychiatric examination may be carried out under compulsion.

Inpatient forensic psychiatric examination is associated with a greater restriction of personal freedom than inpa-

tient examination. Therefore, inpatient forensic psychiatric examination can only be performed if the research necessary for handling matters significant for the case can be carried out in an inpatient setting [18; 19]. And yet, deciding on the type of examination – outpatient or inpatient – is the responsibility of the forensic expert himself/herself. The expert's decision to conduct inpatient forensic psychiatric examination is not subject to judicial review – the legislator has not provided for appeals.

It should be emphasized that there is no provision for inpatient forensic psychiatric examination in civil proceedings. According to Paragraph II of Article 267 of the Civil Procedure Law of the Republic of Latvia [8] as well as According to Paragraph 3 of Article 103 of the Civil Procedure Law of Ukraine [9], if the person subject to the proceedings avoids expert-examination, engaging the public prosecutor, the court may order that the person be subjected to forensic psychiatric examination under compulsion. It must be concluded that it is not possible for the person concerned to deny such examination. The refusal relates only to the refusal to arrive for examination voluntarily, but if refused, he or she will be subject to coercive measures and will be forcibly admitted for the outpatient forensic psychiatric examination. The participation of the prosecutor in the decision-making process ensures the observation of the rule of law, because forced examination restricts the freedom of the person.

Article 283 of the Criminal Procedure Law of the Republic of Latvia [6] provides for the possibility of a suspect, accused, or the person in relation to whom proceedings have been initiated for the determination of compulsory measures of a medical nature may be forcibly placed in a medical institution for the performance of an expert-examination, if the research necessary in a forensic or court psychiatric examination for the solving of matters significant to the case can be carried out only under medical inpatient conditions. A person may be placed in a medical institution for the performance of an expert-examination, on the basis of a decision of the investigating judge or court decision, only if the decision has also been taken on determination of the relevant expert-examination. The decision to be placed in a medical institution for examination is subject to appeal in court.

Under Paragraph I, Clause 5 of Paragraph II of Section 26 of the Criminal Procedure Law of the Republic of Latvia, forensic psychiatric examiners of the court have the power to conduct criminal proceedings on behalf of the State. Paragraph I and Clause I of Paragraph II of Section 33 of the Criminal Procedure Law of the Republic of Latvia provide that the expert of the examination body has the power to conduct criminal proceedings, as well as the expert on behalf of the person directing the proceedings conducts examination if it is necessary to carry out research using specialist knowledge, devices and substances to obtain the information necessary to prove the case. Paragraph 4 of Article 61, Clause 4 of Paragraph I of Article 67 of the Criminal Procedure provide that the person against whom the criminal proceedings have been initiated shall

be required to authorize his/her expert-examination and to issue samples for comparative investigation which are independent of the person's will. Similar provisions are also contained in Article 509 of the Criminal Procedure Code of Ukraine [7].

In view of the foregoing, the examined persons that are subject to criminal proceedings may, in the context of inpatient forensic psychiatric examination, be subject to restrictive measures and non-disclosure if this is necessary for conducting the proceedings and does not conflict with the rights of the persons against whom criminal proceedings have been initiated, but shall not be permitted where this is not justified by the procedural steps. It should be emphasized that forensic psychiatric experts have an obligation and an opportunity to become acquainted with the materials of the criminal proceedings, which may affect the quality of treatment.

Forensic psychiatric examination is a widely applicable means of proof in civil proceedings. The Civil Procedure Law of the Republic of Latvia [8] provides for the performance of examination in cases involving the restriction of a person's legal capacity (Chapter 33 of the Civil Procedure Law). And yet, the categories of such cases are not the only ones when it is essential to ascertain the person's legal capacity. As mentioned above, there is a need for such categories in cases where an action is called to be recognized as void on the ground that the person that has committed it at the time of the transaction or commitment of this action had legal incapacity. Analyzing the norms of the Civil Procedure Law of the Republic of Latvia [8], it has to be concluded that there is no general procedure for determining and conducting forensic psychiatric examination. The issues regarding the limitation of a person's legal capacity are subject to special court procedure, but the procedure of forensic psychiatric examination within the procedure of claims is not specified. In most cases, the claim procedure in litigation are cases of invalidation of the transaction on the ground that the person was legally incapable at the time of the conclusion of the transaction, and in such cases it is the expert's opinion that is crucial for the establishment of the truth. The person's mental state is assessed solely on the basis of other evidence provided in civil proceedings (statements of the parties, witness testimony, written evidence) and without forensic psychiatric examination, i.e. without the involvement of a person with medical expertise, such evidence and assessment are considered incomplete.

As Professor V.Bukovskis has pointed out, the expert is considered to be an objective assistant to the judge in matters where the court lacks knowledge [21]. This demonstrates the need for expertise in much broader issues. It is, however, the prerogative of the parties to request the expert-examination. This fact has also been pointed out by the Supreme Court of the Republic of Latvia, which noted that it is not possible to oblige the parties to take procedural steps, in this case, to request the expert-examination [22].

The essence of forensic psychiatric examination is to ascertain the facts relevant to the case if they require

specialized knowledge which the person who directs the proceedings does not possess. What is to be recognized as an essential fact is not specified in the legal framework, it must be decided on a case-by-case basis. Forensic psychiatric examinations are performed in outpatient settings but also in inpatient medical facilities, when the fact ascertainment is impossible in the outpatient settings. Both types of examination are to be performed in the examination institution and a general medical institution. Within the framework of procedural regulation, forensic psychiatric examination is not identifiable with medical treatment. However, given that some components of forensic psychiatric examination have been linked to treatment by the legislature (the consent of the subject, the location of examination in a medical facility), it cannot be denied that the process of forensic psychiatric examination includes treatment elements. Forensic psychiatrists have a dual status. Namely, they are medical practitioners and are subject to the rules of law deriving from the Medical Treatment Law, and at the same time, they have a special procedural status – an expert who is subject to the rules of criminal and civil law. Different legal frameworks create different legal consequences and, in some cases, hierarchical problems. Similar issues are with the status of the subject of such examination. There is no restriction of personal liberty in civil proceedings, which is why there is no reason to ascertain that the person subject to the examination in civil proceedings is not endowed with the rights of a patient; however, in some cases there is applied a coercive mechanism, namely, forced transfer of a person to an expert-examination. Such forced transportation must not be confused with forcing expert-examination. In civil proceedings, unlike in criminal proceedings, there is no provision for placing a person in a psychiatric medical institution for compulsory examination. In private law, forcing a person into a psychiatric treatment facility would be an excessive restriction of a person's freedom, given that examination in civil proceedings is conducted only at the request of one of the parties, which presupposes voluntary cooperation. At the same time, however, there are downsides to this, as non-cooperation by the person concerned may prevent the truth of the case from being established.

The scope of the patient's rights ends when the implementation of the coercive measures provided for in the criminal proceedings is carried out, which may be applied to the person concerned in order to carry out the procedural act – the expert-examination. Restrictions that do not pertain to forensic psychiatric examination are not permissible.

CONCLUSIONS

Forensic psychiatric examination has several levels of coverage. The expert is an official duly approved by law and is also a doctor. Similarly, the examined person is the person whose duties and rights are determined by the procedural law and is also a patient with a much broader range of rights. These statuses interact. During the forensic

psychiatric examination, the medical practitioner does not perform medical treatment consistently with the Medical Treatment Law of the Republic of Latvia [1]. According to Ukrainian legislation [19], during a stationary forensic psychiatric examination, if there are indications, medications can be used only with the informed consent of the person and under the standards of treatment. However, such use of drugs cannot be considered a full-fledged treatment.

Forensic psychiatric examination is limited at a specific time when the subject under investigation is evaluated using a scientific and legal methodology. The examiner is limited by the task assigned by the person directing the proceedings, namely, the questions posed in the decision ordering the examination, to which the forensic expert must provide answers.

The current regulatory framework does not provide for the procedure by which the subject's medical treatment is conducted during forensic psychiatric examination, nor does it determine the criteria for the admissibility of treatment of the person concerned and the extent of such treatment.

During the examination, which is a long and continued process, the medical practitioner, who, within the specific examination framework, has the expert's procedural status in relation to the subject of the examination, should not carry out treatment of this person. However, in the case of forensic psychiatric examination, it cannot be denied that the subject of investigation may be treated. This treatment is carried out by the same person who conducts expert-examination. In this case, different procedural states of the same person, such as the status of the medical practitioner (doctor) and expert, are merged.

Given that forensic psychiatric examiners must first and foremost have the appropriate qualifications to qualify to carry out expert-examinations, it is understandable that there should in fact be a sign of equivalence between the doctor and the expert, but given that the medical practitioner and the expert have different competencies, it shall not be allowable for the same person to conduct both the treatment and expert-examination of one person in the context of one process.

Within the legal framework, it is necessary to distinguish between the regulation of the examination process and the treatment, while conducting examination. In the first instance, it should be stipulated that forensic psychiatric examination is to be carried out only in a specialized forensic institution where the examination is carried out by an expert who does not provide treatment in any other medical institution. This would help to distinguish the procedural rights of the expert and the doctor and would clearly draw the line between treatment and examination. Secondly, the legal framework must determine the patient's rights as the subject of the examination, since, even in the case of an examination, there shall be no interference with the person's integrity against his or her will.

Subjection to forced forensic psychiatric examination should only be allowed in cases analogous to the provision of psychiatric help without the patient's consent. There should be a clear legal framework in criminal proceedings that is

in line with human rights and guarantees the protection of a person's fundamental rights when placed in a psychiatric institution. This aspect is particularly important because the decision to conduct inpatient forensic psychiatric examination is made by an expert and is not subject to appeal.

Failure of the subject of examination to cooperate with the expert and refusal of inpatient forensic psychiatric examination would be likely to interfere with the clarification of the truth, but is proportionate to the individual's fundamental rights. It should not be forgotten that the expert opinion has no predetermined probative value, as it is evaluated in conjunction with other evidence so that the court does not have only one piece of evidence.

Forced placement for expert-examination in a psychiatric treatment facility is not allowed in civil proceedings. However, the person who is subject to criminal proceedings in the context of inpatient forensic psychiatric examination is permitted to be exposed to the application of restrictive measures and the non-disclosure of information if this is necessary for conducting the proceedings and does not conflict with the rights of the person against whom criminal proceedings have been initiated, and yet it is not allowable unless procedural steps justify it.

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REVIEW ARTICLE

IMPLEMENTATION OF DIGITAL TECHNOLOGIES IN HUMAN RIGHTS TO HEALTHCARE

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The aim: The aim of the study is to examine the blockchain technology in the field of healthcare, to analyze the principles of the European Convention on Human Rights regarding respect for private and family life, home and correspondence, to analyze the key positions of the European Court of Human Rights (hereinafter – ECHR) in the field of human rights to privacy, to analyze the European Union (hereinafter – EU) secondary legislation regarding the supply of medicines, prospects for the blockchain usage in order to protect human rights to privacy and improve the quality of medicines.

Materials and methods: Scientific works that are devoted to the outspread of digital technologies in healthcare, the provisions of the European Convention on Human Rights, the ECHR's practice on the protection of human rights to privacy, the provisions of the EU secondary legislation that regulate the supply of medicines are studied. The methodology of this article is based on comparative and legal analysis techniques and includes system-structural method, method of generalization, method of analysis and synthesis as well.

Conclusions: The blockchain technology in medicine and pharmacology will increase the level of protection of human rights to healthcare quality.

KEY WORDS: blockchain, human rights, counterfeit medicines, quality of medicines

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INTRODUCTION

The current stage of IT development has considerable potential to address the challenges of improving the quality of healthcare services. At the same time, the use of digital technology poses serious challenges for governments to improve the quality of healthcare and pharmaceuticals.

Today when digital technologies become more important, it is no longer possible to imagine our life without blockchain, a fault-tolerant decentralized system, the core principles of which are that each subsequent block in the blockchain contains the hash value of the previous block, and all attempts to change the data recorded on a particular block levels, will cause changes at all subsequent levels and will be noticed by other participants (network nodes). This ensures database integrity verification and a blockchain sequence is formed in which each subsequent block stores the hash value of the previous one [2, 277].

Blockchain has gone far beyond cryptocurrencies, and has spread to all walks of life in society and the state, and thanks to its coordination capabilities and the ability to retain information has become a modern Magna Carta of fundamental social progress. The advantage of blockchain over other storage media is that the data that gets to it can be analyzed while remaining private and unchanged [3, 149-150].

Theoretically, blockchain can be applied to any database and provide reliable storage of information. Blockchain technology has the potential to transform health care, placing the patient at the center of the health care ecosystem

and increasing the security, privacy, and interoperability of health data. This technology could provide a new model for health information exchanges (HIE) by making electronic medical records more efficient, disintermediated, and secure [4].

The technical features of blockchain have been the subject of many research works [2; 4; 5], at the same time, the legal aspects of this technology were fragmented, and were mainly devoted to the legal regulation of blockchain usage in the context of fintech industry in different countries of the world [6; 7; 8].

The question of protecting human rights on privacy, including the right to personal information, non-disclosure of medical information, and human rights to quality medicines, remain relevant today. At the same time, it should be noted the complete absence of theoretical elaboration of the problems of legal regulation of relations with the use of blockchain technology in this field.

Tracking drug chains, storing patient records, health insurance are the part of incomplete list of options to use distributed database technology in healthcare. The research of legal issues arising from the use of blockchain technology in the field of human rights is fragmented.

THE AIM

The aim of the study is to examine the blockchain technology in the field of healthcare, to analyze the principles of the European Convention on Human Rights regarding

respect for private and family life, home and correspondence, to analyze the key positions of the European Court of Human Rights (hereinafter – ECHR) in the field of human rights to privacy, to analyze the European Union (hereinafter – EU) secondary legislation regarding the supply of medicines, prospects for the blockchain usage in order to protect human rights to privacy and improve the quality of medicines.

MATERIALS AND METHODS

Scientific works that are devoted to the dissemination of digital technologies in healthcare, the provisions of the European Convention on Human Rights, the ECHR's practice on the protection of human rights on privacy, the provisions of the EU secondary legislation regulating the supply of medicines are studied. The methodology of this article is built upon comparative and legal analysis techniques and includes system-structural method, method of generalization, method of analysis and synthesis as well.

REVIEW AND DISCUSSION

As it mentioned in Deloitte's 2019 Global Blockchain Survey, 40 percent of health professionals see blockchain as a top priority. In addition, the global healthcare market is expected to spend \$ 5.61 billion by 2025, according to a report by BIS Research «Global Blockchain in Healthcare Market- Analysis and Forecast, 2017-2025». The adoption of the blockchain technology will help save the industry up to \$ 100-150 billion per year by 2025 in data breach-related costs, IT costs, operations costs, maintenance and staffing costs, and through a reduction in frauds and counterfeit products [9].

The first launch of blockchain took place in 2009 and was only associated with cryptocurrency, but since 2014 other blockchain opportunities have been discussed greatly. In particular, the attention to the potential of a decentralized approach and the use of such a consensus mechanism whereby the final state of database accounting system would be well protected against change was drawn. Blockchain, as a way of organizing data storage, has enabled accounting systems with multiple independent validators to achieve not only secure synchronization of databases, but also the invariability of this data, subject to some assumptions [2, 274].

The formidable innovation introduced by blockchain is that the network is open and participants do not need to know or trust each other to interact: the electronic transactions can be automatically verified and recorded by the nodes of the network through cryptographic algorithms, without human or any other intervention (banks, financial institutions or other organizations). Blockchain has become «a disruptive innovation with a wide range of applications, potentially able to redesign our interactions in business, politics and society at large» [10, 45].

In healthcare, the issue of information management is very topical. Healthcare providers work daily with data that

requires careful handling, anonymity, and correct transfer of information. It also requires specific rules regarding access to medical data, a specific algorithm for managing rights, developing common standards for entering and handling “big data”.

The protection of personal data, including medical information, is a fundamental feature of the right to privacy [11]. Medical data are subject to both Article 8 (1) of EU Directive 95/46 / EC on data protection [12] and Article 6 of Council of Europe Convention No 108 [13], which stipulate in particular that personal data concerning health, may not be processed automatically unless domestic law provides appropriate safeguards.

To protect personal information stored in automated databases, appropriate security measures for preventing their accidental or unauthorized destruction or accidental loss, as well as preventing their unauthorized access, alteration or distribution of such data should be taken.

In the thematic report “Health-related issues in the case-law of the European Court of Human Rights” the Court has acknowledged that the protection of personal data, including medical information, is of fundamental importance to the enjoyment of the right to respect for his or her private and family life guaranteed by Article 8 of the Convention [14].

In *I. v. Finland* the applicant, an HIV-positive nurse, suspected that unauthorised persons had accessed her medical records. While the strict application of domestic law would have constituted a substantial safeguard in her case, the system at the hospital made it impossible to clarify retroactively the use of patient records or to determine whether information contained on the applicant and her family had been given to or accessed by unauthorised parties. Moreover, at the material time the records could also be read by staff not directly involved in her treatment. Although the hospital had subsequently taken ad hoc measures to protect the applicant against unauthorised disclosures by restricting access to treating personnel and registering her under a false name and social-security number, this had come too late. What had been required in the applicant's situation was practical and effective protection to exclude any possibility of unauthorised access occurring in the first place. The Court therefore found a violation of Article 8 [15].

According to the requirements of Article 8 of the Convention, the use of samples of human biological materials (cell samples, DNA data, fingerprints) in criminal proceedings should also be mentioned.

Rules for the use and storage of biological samples of human tissues (including DNA data) in the investigation of crimes are set out in Council of Europe Committee of Ministers Recommendation No. R (92) 1 of 10 February 1992 “On the use of analysis of deoxyribonucleic acid (DNA) within the framework of the criminal justice system” [16]. However, in spite of the high level of regulation of the problem, there is the concern in society about human rights abuses during the creation and use of DNA databases [17, 151].

Currently, there is no standard in Ukraine for the storage and transmission of medical data. In June 2019, the Ministry of Health of Ukraine proposed a draft decree of the Cabinet of Ministers of Ukraine "On approval of the Health Informatization Concept of Ukraine" [18]. The concept presented contains a strategic plan for the development of the e-Health system, basic principles of its operation, necessary legislative changes and prerequisites for the investment attractiveness of the system.

It is envisaged to solve the problem of inadequate level of efficiency and transparency of health care, which leads to inefficient use of resources, by ensuring timely and reliable information for the state institutions involved in the management of the health care system, by using the benefits of handling «Big Data» and intelligent systems to predict health care needs, by planning resources in the industry and engaging the patient in their own health care, by monitoring the quality of the medical services received by providing access to and disposal of their own medical data.

Data fragmentation will be avoided due to the electronic system which will allow to store patients' data in a secure cloud storage facility instead of paperwork and which will allow doctors of different medical institutions to have access to the necessary information.

It is stated in the analytical note to the Cabinet of Ministers of Ukraine as for the project, the process of electronic health system introduction in Ukraine is characterized by lack of compatibility of information systems in the field of health care, lack of a unique identifier for patients, imperfection of information infrastructure and interaction between general registers, imperfection of a number of registers, absence of specialist staff for automation and change management, lack of computer and networking equipment in healthcare facilities, etc. [19].

Within 2017–2018, a number of systematic changes in the field of health informatization took place, a number of by-laws regulating the activity of the electronic health care system and institutions were implemented – Ministry of Health, National Health Service of Ukraine, State Enterprise "Electronic Health".

E-government is being introduced in Ukraine as a modern tool of the effective interaction between the state, civil society and business, which is intended to fully implement the rights and interests stipulated by the legislation, to use effective mechanisms of rights and freedom protection, to influence the actions or inactivity of state authorities and to hold their officials responsible in cases established by law [20, 52] and the possibility of access to the system of interaction of state electronic resources "Trembita" is being expected. Activities in this field are conducted by the State Agency for Electronic Governance of Ukraine.

Undoubtedly, it is difficult to overestimate the importance of the proposed concept for the future of e-health and medical reform. At the same time, its imperfection should be mentioned. One of the vulnerabilities of the medical field, and the concept, unfortunately, does not provide a solution to this problem, is the protection of patients' personal data and the issue of responsibility for

their accessibility and preservation. It is also unclear what the concept of security is, what the means of patient authentication are.

According to the Article 3 of the European Charter of Patients' Rights, every person has the right to receive full information on health status, medical care, including medical interventions, the possibilities and conditions of use of the results of medical care and all available modern technologies [21].

Blockchain technology opens up very interesting and promising storage, transmission and data management capabilities. Among the blockchain projects which have been already implemented the following ones can be pointed out. *IRYO* is a platform for storing and controlling patients' electronic medical records based on blockchain EOS. *CareX* is a project that develops through healthcare payments through its CareX token. Increased demand for medical tourism requires cross-border transfers or the use of large amounts of cash. The project solves this problem by using CareX's internal cryptocurrency. Token owners can store their medical information in the system, completely controlling their privacy and making them available as needed. The platform provides support for a chatbot with artificial intelligence that can make previous diagnoses. *SmartHealthCareToday* is a platform for storing personal medical data, integrating EHR (Electronic Medical Card) and PHR (Personal Medical Card) with information about a patient's life, activity, and regular measurements of medical parameters such as blood pressure and blood glucose. Access to health data through in-app users can be tailored to each patient by the program. Brdt.pro lab has conducted research of blockchain projects focused on health care work and has counted over 20 medical data storage and management solutions. Today it is the most popular field of development in medicine [22].

According to WHO information, one in ten medicines distributed in low- and middle-income countries is either non-conventional or counterfeit [23]. The current model of state regulation of the medicines supply in Ukraine does not provide medicines of the proper quality, which does not only cure but can also cause serious illness or even patients death.

The EU legal regulation of the medicines circulation should include the following: Directive 2001/83 / EC on the Community code relating to medicinal products for human use [24], Directive 2004/23 / EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells [25], Directive 2003/94 / EC laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use [26], Regulation No 1394/2007 on advanced therapy medicinal products and amending Directive 2001/83 / EC and Regulation No 726/2004 [27], Regulation No 141/2000 on orphan medicinal products [28], Ordinance No. 1901/2006 on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004 [29].

As the rules of the EU's "secondary law" acts are not the acts of direct effect in Ukraine, their implementation is necessary therefore. To address this issue, national legislation should be harmonized with EU law in accordance with the EU-Ukraine Association Agreement and its annexes.

The implementation of the requirements of the above mentioned documents of the European Union is intended to facilitate the regulation of legal relations in the sphere of circulation of medicines related to the creation, issuance of trade licenses, production, import, wholesale and remote trade, quality control of medicinal products, definition of these rights and obligations, as well as the powers of public authorities in this area.

As defined in point 33 of Section I of Directive 2001/83 / EC, a falsified medicinal product is any medicinal product with a false representation of:

- (a) its identity, including its packaging and labelling, its name or its composition as regards any of the ingredients including excipients and the strength of those ingredients;
- (b) its source, including its manufacturer, its country of manufacturing, its country of origin or its marketing authorisation holder; or
- (c) its history, including the records and documents relating to the distribution channels used [24].

Global trends in medicines counterfeiting counteraction combine effective mechanisms for combating the spread of counterfeit medical devices at international, European regional and national levels [30].

The effectiveness of the drug control mechanism and the liability of individuals for drug law offenses can be significantly improved by the use of blockchain technology, which can be a solution to improve data security, integrity, functionality and, of course, the security of the pharmaceutical supply chain. This technology can be used to overcome the problem of identity identification, sources of origin, as well as the history of drug distribution, as it will allow tracing the entire supply chain, preventing falsification as such [31].

Thus, blockchain is a real revolution in the healthcare industry as it is well suited to protect all aspects of distributed networks and helps to record, store, sort and transmit data in such a way that they remain relevant and unchanged. Once the supply chain (medicines) becomes visible and transparent with the help of blockchain, it will be possible to trace the origin of the medicines (the quality of the medical services provided). Beneficiaries of blockchain technology will be healthcare facilities and pharmaceutical companies by reducing the costs of counterfeit products and patients receiving quality medicines.

The use of blockchain technology not only minimizes the risks of supplying counterfeit medicines to patients, but also ensures transparency of delivery and reduction in price of the final product for patients [32].

Blockchain issues have been developed in the works of Swan Melanie [3], Arvind Narayanan, Joseph Bonneau, Edward Felten, Andrew Miller, Steven Goldfeder [5], Atzori M. [10], who have focused on blockchain as the latest digital technology capable cause profound changes in society and are, to some extent, debatable. In particular, blockchain

and decentralized platforms are explored as hyperpolitical tools capable of managing large-scale social interactions and abolishing the traditional vertical of power as such. The analysis highlights the risks associated with the dominance of private interests in distributed ecosystems, which could lead to the emergence of a global, stateless society.

The introduction of blockchain technology was investigated in the work of V. Pashkov, which emphasized the need for adequate legal support for the full involvement of this technology in the field of pharmacy [32].

The vulnerability of human rights to the formation of DNA databases is one of the objects of study by M. Goc, H. Dębowska [17]. In turn, the relationship between the privacy of information contained in human DNA and the public interest in using such information in the investigation of crimes is a subject of debate in R. Erbaş's work [33].

CONCLUSIONS

Global health trends aim to improve the protection of human rights to respect for privacy and the quality of medicines. The analysis of EU legislation shows that the protection of human rights for quality healthcare is provided at the conventional level, on the basis of the European Convention for the Protection of Human Rights and Fundamental Freedoms, as well as at the level of secondary law acts, with further implementation in the national legislation of the states.

Health informatization allows us to see the prospects of blockchain using to protect human rights in the fields of medicine and pharmacology. Overcoming the problems of storing and managing patient data, tracking the origin of medicines, maintaining data consistency while designing medicines are just some of the challenges that blockchain has successfully handled.

The use of artificial intelligence, big data analytics and blockchain can work together to ensure efficiency and accurate results in the medical and pharmaceutical environment, and thus increase the level of human rights protection for quality healthcare.

Further research are in need to assess the possible risks and costs associated with blockchain, and to take into account the legal aspects of technology implementation and to bring them into line with current legislation of countries.

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REVIEW ARTICLE

NON-NECROTIZING INFLAMMATORY GRANULOMAS (N-NGS) IN THE COURSE OF INFLAMMATORY BOWEL DISEASE – IMMUNOLOGY AND CLINICAL MANIFESTATION OF INTESTINAL AND RESPIRATORY N-NGS

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ABSTRACT

The aim: The article describes and summarizes the immunological pathomechanisms controlling the development of non-necrotizing granulomas in the course of non-specific inflammatory bowel diseases (IBD) in lungs and intestines; it also reviews the possible clinical correlations between the processes in the gastrointestinal and respiratory tracts based on the example of Crohn's disease (CD) and non-specific inflammatory bowel disease (IBC).

While the dominant cell subpopulation in ulcerative colitis (UC) is Th2, which produces interleukins IL-4, IL-5, IL-6, IL-10 and IL-13 and Th17 cells; CD characterized by the Th1 cell subpopulation and macrophages predominate, producing IL-23. These are considered to be the key factors crucial for the occurrence of chronic inflammation. Another important causative factor of non-specific inflammatory bowel diseases and granulation is the expression of CD40/CD40L proteins on activated T-cells, i.e. type 2 transmembrane proteins similar to TNF- α . However, the interactions between gastrointestinal neuroendocrine peptides/amines (NEPA) and the immune system are believed to have a significant influence on the pathophysiology of non-specific inflammatory bowel diseases and non-necrotizing granulation. The key functions of the immune response of the gastrointestinal tract are managed by the neuroendocrine regulatory system (NES) whose activities govern the production of various hormones including chromogranin/secretogranin, serotonin, vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), substance P, somatostatin or ghrelin.

KEY WORDS: Crohn's disease, inflammatory bowel diseases (IBD), non-necrotizing granulomas (N-NGs), ulcerative colitis (UC)

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INTRODUCTION

Although the etiology of non-specific bowel inflammation remains unclear, it is known to involve persistent bowel infections, and mucosal barrier defects, as well as various immunological, genetic and environmental factors. It has been found that non-necrotizing granulomas commonly occur in the intestines and the lungs as characteristic lesions during the course of non-specific inflammatory bowel diseases. Immune system dysfunction and mucous membrane disorders play a significant role in this type of granulation: one aspect of the chronic inflammation underlying non-specific inflammatory bowel diseases (IBD).

Non-specific IBD is a term defining a group of inflammatory conditions of the gastrointestinal tract. The two most common, and most frequently described, conditions representing this group are non-specific ulcerative colitis (UC) and Crohn's disease (CD). Although the precise cause remains unknown, the etiology of the diseases is conditioned by genetic and environmental factors, as well as the immune system of the patient [1, 2].

It is estimated that 1 to 1.5 million Americans and 2.5 to 3.0 million Europeans suffer from non-specific IBD, as do hundreds of thousands of individuals around the rest of the world. UC is more widespread than CD [3] with the ratio of UC to CD being found to be as high as 15:1, identified in Poland in 2005 [4].

The annual incidence rates of UC per 100 000 individuals vary worldwide: from 6.3 in Asia and the Middle East, through 19.2 in North America to 24.3 in Europe. Similarly, the incidence of CD ranges from 5.0 per 100 000 individuals in Asia and the Middle East, to 12.7 in Europe and 20.2 in North America [5]. In Poland non-specific IBDs affect about 0.1% of the population; however, the number is increasing [6]. One study indicates the overall annual incidence to be 2.7 per 100 000 children in Poland [7].

Non-specific IBD occurs most frequently in white populations; however, its incidence has grown in other ethnic groups [8, 9]. Considerable variation exists between populations regarding its occurrence: for example, IBD occurrence is three times higher in the Jewish population than in some other non-Jewish populations [8].

THE AIM

Recent studies have revealed a relationship between the occurrence of CD and the presence of mutations in such genes as ATG16L1, CX3CR1, IL-23R and MMP3. Additionally, blood serum-producing cells, growth factors, miRNA and microbiological antibodies are also associated with lesions in patients with CD [10].

REVIEW AND DISSUSION

ETHIOPATHOGENESIS

The etiology of non-specific IBD remains unknown. The disease process involves persistent bowel infections and mucosal barrier defects, as well as immunological, genetic and environmental factors [11].

Family inheritance suggests that the disease might have a genetic basis. Indeed, several genes indicating susceptibility to the disease have been identified, as well as 163 positions of nucleotide polymorphisms in the genome. Susceptibility to the disease depends on the mutation of the NOD2 protein gene, CARD15, located on chromosome 16. The gene helps maintain the balance between the intestinal microbiota and the host immune response to inflammation by activating a pro-inflammatory transcription program; this system participates in the production of pro-inflammatory proteins and increases the intensity of the inflammatory response and other immunology pathways, *inter alia* by regulation of macrophage activation in response to bacterial liposaccharides [12]. The presence of two alleles of the mutated NOD2 gene increases the risk of developing CD by even 20-40 times [2].

Patients presenting with non-specific IBD also display differences in the quality and amount of their bacterial flora. A crucial role in the development of inflammation may be played by certain strains of *Escherichia coli* and *Bacteroides vulgatus* [1].

Smoking reduces both the risk of occurrence and the severity of UC, – however, it also increases the risk of CD and worsens its course [1, 2].

A significant factor in the pathogenesis of non-specific IBD is dysfunction of the immune system of the mucous membrane. It is believed that out of the various inflammatory cells of the intestines, mucosal T CD4 + cells play a central role in both the induction and maintenance of chronic inflammation through production of pro-inflammatory cytokines. While in physiological conditions, the lamina propria of the intestinal mucosa contains large numbers of various immune cells, such as T, B, NK, NKT cells, dendritic cells, macrophages, mastocytes, neutrophils, eosinophils and stromal cells (fibroblasts), in the case of inflammation, a large number of activated immune cells are able to penetrate the intestinal mucosa. These cells produce numerous inflammatory mediators; in addition, fibroblasts and the endothelial cells of intestinal mucosa vessels also present high levels of chemokines, selectins (e.g. E selectins) and intracellular adhesion molecule-1 (ICAM-1 or CD54). These factors interact with the leukocytes circulating in the blood, making them migrate to the intestinal mucosa and promoting a local inflammatory response [13].

The courses both of UC and CD are characterized by increased activation of T CD4+ (Th) lymphocytes. Activated T lymphocytes produce increased amounts of cytokines, which further progress the course of the immune response [1]. In patients suffering from CD, CD4+ lymphocytes are able to produce large amounts of pro-inflammatory

cytokines bound to Th1 / Th17 (e.g. interferon-gamma – IFN- γ , tumour necrosis factor – TNF and interleukin 17A – IL-17A) [13].

In contrast, in patients with UC, the Th2 subpopulation dominates. These cells produce interleukins IL-4, IL-5, IL-6, IL-10 and IL-13; they also produce Th17 cells, which secrete various interleukins including IL-17A. An imbalance between the levels of pro-inflammatory cytokines, such as IL-12, IL-18, IL-21 and IL-23, and anti-inflammatory cytokines, such as TGF- β , IL-10, IL-25, IL-33 and IL-37, results in inflammation of the intestinal mucosa [13]. Although it was previously believed that IL-12 was the key factor initiating the inflammatory response by Th1 in CD, more recent studies in 2006 suggest that it may be IL-23 [14][15].

UC itself is triggered by a Th2-like response, the main product of which being natural killer (NK) cells which generate IL-5 and IL-13 [16].

During mucosal inflammation in patients with non-specific IBD, the titre of regulatory lymphocytes (Foxp3), T-cells (Treg) and FoxP3-IL-10+ CD4+ decreases. This results in part of the intestinal mucosa displaying decreased immunity, as well as increased support for the local immune response, leading to damage to the intestinal mucosa [13].

IL-22 is a member of the IL-2 cytokine family, which is produced mainly by CD4+ T- lymphocytes. It shows a pleiotropic ability to regulate differentiation and functions of T-cells and strengthens antigen-specific clonal expansion. IL-21 is also associated with the immune response through Th2 and plays a role in inhibiting the differentiation of naive Th-cells into Th1 cells producing IFN. It promotes the maturation and activation of human NK cells, triggers other biological functions in B-lymphocytes and regulates the differentiation and production of antibodies.

Elevated amounts of IL-21 are observed in the intestines of patients with non-specific IBD. In addition, it increases the expression of transcription factors bound to Th1 and IFN- γ in T and NK cells and enhances the cytotoxic activity of NK cells. IL-21 also inhibits peripheral Treg differentiation and makes CD4+ T-lymphocytes resistant to immune suppression by Treg [13]. IL-13 is an additional pathway for the production of pathogenic Th-17 cells. The combination of TGF-beta and IL-21 also inhibits Foxp3 expression in naive T cells via IL-17 induction, which suggests that IL-21 is able to regulate the mutual pathway of development of Treg and Th17 cell generation [17].

Therefore, IL-21 may damage the intestines via a range of signalling pathways, and its neutralization may be of value in the treatment of non-specific IBD.

IL-23, produced mainly by macrophages, is also known to be one of the key cytokines in non-specific IBD and to play an important role in promoting chronic bowel inflammation.

Overexpression of IL-23 in the intestinal mucosa inhibits the production of IL-10 [18][13]. IL-10 induces the Th-2 response; this plays a crucial role in adaptive immunity by inducing the production of specific antibodies to eliminate invading microorganisms and absorb their products [13].

IL-10 is one of the most effective cytokines responsible for the regulation of the immunological system. Its reduced expression in non-specific IBD weakens the host defence by decreasing the production of IgA in the intestines [18].

Both Th17 lymphocytes and the cytokines they secrete, for example IL-17, IL-21, and IL-22 [16], also play important roles in the activity of non-specific IBD through their associated damage to the intestinal mucosa.

Another important role in non-specific IBD is also played by CD40-ligands and CD40L (CD-154). CD 40/CD40L are type W2 membrane proteins similar to TNF-alpha. CD40 undergoes transient expression in activated T cells and is an important agent in B-lymphocyte production, as well as the activation and differentiation of monocytes and dendritic cells. Both CD and UC are characterized by local overproduction of cytokines such as TNF-alpha and an inflow of activated lymphocytes resulting in inflammation of the mucosa of the gastrointestinal tract [20]. Cytokines through their respective influence on activated T-lymphocytes and CD40 play important roles in the proliferation, differentiation, and production of immunoglobulins and in the isotype of the B lymphocytes [21-23]. The expression of CD40 on monocytes, macrophages and dendritic cells is related to the role it plays in the signalling pathway in the cellular immune response.

Adhesion of CD40 on monocytes and macrophages induces the production of pro-inflammatory cytokines and prevents apoptosis. *In vivo* tests have demonstrated the importance of CD40-CD40L interaction in triggering humoral immune responses, stimulating and activating specific T-cells and inducing temporary activation of macrophages. It also plays a significant part in immune responses against intracellular parasites, which are mediated by the activation of macrophages via T-lymphocytes [20]. CD40 stimulation initiates a complex, innate immune cascade which boosts the development of the systemic and local immunological pathology of the intestine. It has been found that cytokines such as TNF-alpha and IL-12 are preferentially associated with the systemic immune response; in contrast, IL-23 activity is mainly associated with the local immune response in the intestines and plays an insignificant functional role in systemic immunologic activation. It is likely that anti-CD40 application induces the activation of myeloid cells, which depends on the auto-crine activation loop controlled by IL-12. Following CD40 ligation, myeloid cells produce a series of inflammatory cytokines, including IL-1-12, which leads to the production of TNF-gamma by NK and other cells. This, in turn, may be repeated by the activation of myeloid cells, resulting in increased levels of pro-inflammatory factors including IL-12, TNF-alpha, MCP-1 and IL-6 [24], thus inducing inflammation of the mucosa and the deeper layers of the intestinal wall in non-specific bowel diseases.

Studies indicate that the Th9 cells producing IL-9 play a significant role in non-specific IBD, particularly UC. They develop out of naive T-lymphocytes, which transform into Th9 under the influence of IL-4 and TNF-alpha [25]. Studies conducted on mouse models indicate that IL-9 is involved

in triggering inflammation of the intestinal mucosa. No increase in IL-9 has been observed in the intestines of patients with CD.

The abundance of the IL-9 receptor in the intestinal epithelial cells of patients with UC suggests that IL-9 impairs the proliferation of the intestinal epithelium and the process of healing damage resulting from chronic inflammation [26].

DEVELOPMENT OF GRANULOMAS

THE PROCESS IN THE INTESTINES

The production of IL-9 may be induced by IL-33: a cytokine which, in cases of UC, is intensively secreted by the epithelial cells of the colon under the influence of inflammatory epithelial cells [27, 28]. The immunologic and pro-inflammatory processes occurring in the course of non-specific IBD give rise to granulomas and, in the case of CD, non-necrotizing granulomas. They are characteristic for this condition and occur in about 40-50% of patients. They comprise epithelioid histiocytes or epithelioid cells and small amounts of multinucleated giant cells and T-lymphocytes. They are composed of epithelioid macrophages and other inflammatory cells, and an extracellular matrix surrounded by the aforementioned lymphocytes. As opposed to other granulomatous diseases, granulomas in CD are small and usually single [29].

It is believed that an important aspect of the pathophysiology of non-specific inflammatory bowel disease is the correlation between neuroendocrine peptides/amines (NEPA) of the gastrointestinal tract and the immune system [30]. The neuroendocrine regulatory system (NES) is responsible for fulfilling the major functions in the immune response of the gastrointestinal tract. The NES is composed of two parts: endocrine cells of the gastrointestinal (GI) tract in the mucosa and the enteric nervous system (ENS). GI cells are found between mucosal endothelial cells directed towards the lumen of the gastrointestinal tract and produce large amounts of hormonal peptides. Mature GI cells may produce as many as seven different hormones such as chromogranin/secretogranin, serotonin, vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), substance P, somatostatin or ghrelin [31]. In the intestines, they play the role of immunomodulators. Of the neuropeptides, serotonin, NPY and substance P induce granulation, whereas neuropeptides, such as granin-derived peptides, VIP, somatostatin and ghrelin, reduce granulation.

Granin-derived peptides are believed to have an anti-inflammatory effects; this acts by decreasing the density of lymphocytes in inflammation sites and reducing the pro-inflammatory activity of both lymphocytes and monocytes by inhibiting the secretion of IL-5 and IL-6 [32]. Serotonin is an important peptide secreted by mast cells, macrophages, monocytes and T cells, which takes part in inflammation processes in the intestines. It promotes the activation of lymphocytes, whose proliferation protects NK cells and auxiliary T cells against degradation, reduces

immune cell (IC) apoptosis and increases T lymphocyte proliferation. Serotonin is generally considered to be a pro-inflammatory amine, as serotonin cells are capable of expressing IL-13 [30].

VIP is produced by neurons, endocrine cells and ICs. It is thought to be the main neuropeptide regulating the immune system, thus playing a significant role in inflammatory disturbances and acting as an important anti-inflammatory agent. VIP inhibits the production of pro-inflammatory cytokines such as TNF- α , IL-6, IL-12, iNOS and promotes the production of anti-inflammatory cytokines such as IL-10 [33].

NPY acts like hormones, neurotransmitters and neuro-modulators. NPY is produced by cells such as lymphocytes, macrophages and dendritic cells during the inflammation process [34]. Substance P is a neuropeptide, a member of the tachykinin family. It is secreted by nerve fibers, i.e. intestinal efferent neurons, widely distributed on the walls of the gastrointestinal tract. It plays a significant role in the migration of intracellular ICs such as neutrophils, macrophages and T lymphocytes. Substance P is considered to be one of the main pro-inflammatory mediators in the gastrointestinal tract [30].

Somatostatin stimulates the proliferation of B-lymphoblasts and increases production of immunoglobulins; it also inhibits the activity of T-lymphocytes and granulocyte proliferation, and reduces the secretion of pro-inflammatory cytokines such as INF- γ [35, 36]. Ghrelin is a peptide which occurs mainly in endocrine cells of the gastric mucosa. It controls consumption of food, energy homeostasis and gastrointestinal motility. It also mediates immune responses and infections. It has an anti-inflammatory function by modulating the secretion of pro-inflammatory cytokines from stimulated macrophages [37].

THE PROCESS IN THE LUNGS

Non-necrotizing granulomas occur in various disorders such as sarcoidosis, CD common variable immunodeficiency disorders (CVID) and cryptococcosis, among others, and may also be present in tuberculosis [37]. Patients with CD often display a pulmonary epithelioid-like non-necrotizing granuloma with a monocyte infiltrate composed mainly of lymphocytes, plasmatic cells, neutrophils and some eosinophils [38, 39]. They occasionally present areas of central necrosis [40]. They are described as sarcoid-like granulomas since they share the same histological and immunological features and, most probably, the same development mechanism [41, 42, 38]. They comprise a central zone with CD4+ T-lymphocytes and the marginal zone with B lymphocytes and CD8+ T-lymphocytes. Non-necrotizing granulomas develop from a non-specific cellular response to unidentified antigenic factors [43]. The major role in the process is played by disturbances in the number of T-reg cells. In a healthy intestine, the intestinal epithelial cells (IECs) serve as a functional barrier, while antigen-presenting cells (APCs) balance the oral immune tolerance. In non-specific IBD, this tolerance declines due

to a general defect in the mucosal immune system: the balance between the functional barrier (including IEC impairment) and the innate and acquired immune response is disturbed. Such defects result in impaired communication between epithelial cells and lymphocytes, and increase disturbances between T-reg cells and CD4+T- lymphocytes, leading to chronic inflammation of the mucosa (also in the respiratory system). The intestines serve as an immunomodulator between the immune system and the epithelial cell barrier found in all the systems, as well as in the respiratory tract, which may result in the development of non-necrotizing granulomas [39]. Granulomatous processes in the lungs have a genetic basis, also common to IBD. The mutation in the NOD2 gene modifies a systemic response of the respiratory and gastrointestinal track to external factors. The NOD2 gene acts as a pattern recognition receptor (PRR) – a receptor recognizing the element of the bacterial cell wall, in particular muramyl dipeptide. This interaction is crucial for a proper response to the infection of the intestinal mucosa. A defective polymorphic variant of the gene occurs in about 15% of patients suffering from CD and is a risk factor for a more severe course of the disease. The same variant of the NOD2 gene is present in patients with a history of severe, chronic sarcoidosis. The significance of the aforementioned correlations becomes even more pronounced when we consider the fact that both the gastrointestinal and respiratory tracks have a common organogenetic origin, they derive from the primitive gut tube. On the basis of the presented data, the hypothesis has been fostered that this common origin of both tracks lies behind the similar lesions in these two systems in the course of IBD (Table 1) [44].

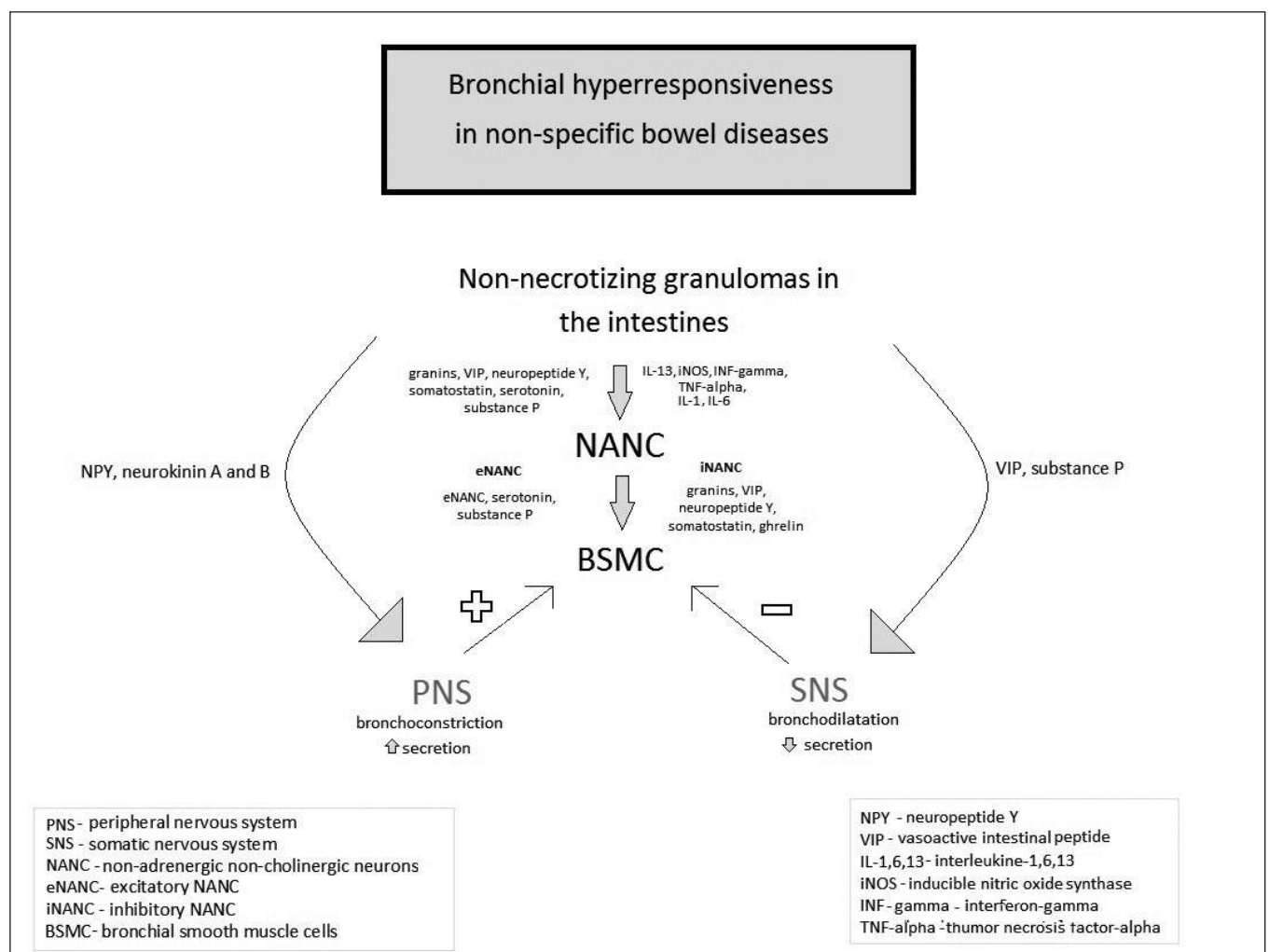
RESPIRATORY TRACT COMPLICATIONS

Extra intestinal manifestations of non-specific IBD in the lungs may cause, *inter alia*, diseases of the bronchi, pulmonary parenchymal diseases, thromboembolism, pleural disease, colobronchial fistula and abnormalities in pulmonary function tests. One of the symptoms of respiratory tract disease in CD is bronchial hyperresponsiveness, whose pathomechanism may involve subclinical inflammation of the respiratory tract triggered by several types of inflammatory cells such as macrophages or lymphocytes, as well as epithelial damage, microbleeding and mechanisms of the autonomic nervous system [49]. Patients with non-specific IBD and congestion of the mucous membrane, these cells and phenomena can typically be observed on bronchofiberscopic images.

Purulent bronchitis is a commonly occurring condition manifested mainly by chronic expectoration of purulent sputum. Tracheal stenosis develops as a result of massive submucous fibrosis of inflammation lumps (granulomas) or peripheral infiltration of the mucosa. Bronchial biopsy reveals overgrowth of the mucosa, thickening of the basement membrane, angiectasis and cellular infiltrations composed of granulocytes, T-lymphocytes and B lymphocytes and non-specific chronic inflammatory infiltrations. Nevertheless, the most typical symptom observed in the

Table I. A comparison of non-necrotizing granulomas of the lungs with those of the intestines. Own devised material based on [45-48]

Non-necrotizing granulomas		
	in the lungs	in the intestines
SIMILARITIES	- etiology not fully known - presence of not layering epithelioid granulomas	- both epithelia are equally prone to lesions caused by an unidentified humoral factor
	- infiltration from lymphocytes, plasmatic cells, neutrophils and some eosinophils - accumulation of T CD4+ lymphocytes at sites of the disease activity	- shared embryological origin of the background for granulomas in the intestines and lungs – the primitive foregut, the so-called gut-lung axis - the background for non-necrotizing granulomas in the intestines and lungs is characterized by similar antigens
DIFFERENCES	- enlarged regional lymph nodes	- lymph nodes are not enlarged as intensively as in the gastrointestinal tract
	- granulomas may develop out of a background which is not affected by inflammation – found on tissues where chronic inflammation process does not exist	- granulomas develop out of a background changed by inflammation – found on tissues where exist chronic inflammation process
	- histopathological image may reveal a large amount of dendritic cells	- a smaller amount of dendritic cells

**Fig. 1.** The immunological mechanisms of bronchodilation and bronchoconstriction in bronchial hyperresponsiveness Based on [58]

fluid obtained by bronchoalveolar lavage (BAL) in patients with non-specific IBD is lymphocytosis. However, if a discharge is found in BAL, mostly from the trachea and bronchi, then the fluid may be dominated by neutrophils.

The most characteristic presentation of small airway disease accompanying non-specific IBD is bronchiolitis. Non-necrotizing granulomas composed of epithelial, multinucleated giant cells and dispersed mononuclear



Fig. 2. CT image of lung granulomas in the form of ground glass opacities.



Fig. 3. HRCT image of lungs displaying non-necrotizing granulomas in the form of lumps.



Fig. 4. CT image of lungs displaying chronic bronchitis: a thickening of the bronchial walls can be seen to the lower lobes, medial lobe, and segments of the left lung lingual.

Photographs – material from own collection

cells surrounding the small bronchioles were analyzed in patients suffering from CD. Bronchiolitis related to UC is typically diffuse, fibrotic and sclerosing, with a severe course, and was found to display non-granulomatous inflammation [44]. For many years, studies have postulated both acute and chronic respiratory manifestations in non-specific IBD; however, the fact that they occur more rarely than in other organs contradicts the hypothesis that the lungs and intestines share the same embryological origin and that they present a similar susceptibility to immunologic diseases [50]. This contradiction may be accounted for by the fact that the epithelia of the airways contain a system that is autonomous for cells where active cilia both capture harmful substances entering the airways and initiate the defence mechanism which eliminates the harmful compound. Although the lungs also have to deal with microbiological and xenobiotic exposure, its level is not as great as that experienced by the intestinal epithelium. This may explain why the lungs are comparatively rarely affected in cases of CD [51]. Pharmacotherapy is another factor which causes further complications. Reactions such as dyspnea, coughing, impaired diffusing capacity of the lungs and interstitial pneumonia have been described in the immune complexes of the lungs during application of mesalazine, aurum salts, arechin or sulphasalazine [52, 53]. It has been demonstrated that the toxicity of sulphasalazine may mimic clinical granulation with vasculitis (Fig. 1) [54]. Sulphonamides, e.g. sulphasalazine or mesalazine, containing the residue of 5-aminosalicylic acid (5-ASA) are basic medicines used in pharmacotherapy of non-specific inflammatory bowel diseases. Drug-related adverse reactions are pulmonary complications and a characteristic radiological picture, however, their occurrence is very low. Both sulphasalazine and mesalazine can provide a picture of organizing pneumonia (OP) and their clinical signs and symptoms, as well as radiological and histopathological changes are the same as in idiopathic OP. Typical for OP peripheral interstitial radiopacities with air bronchogram of a changing location are visible on a chest X-ray. Histological examination reveals interstitial inflammation and polypoid plugs of the developing fibrous tissue filling the terminal bronchioles and parts of the alveoli. High resolution computed tomography (HRCT) depicts mainly peribronchially localized parenchymal thickening areas and small areas of ground glass opacity (Fig. 2). After using sulphasalazine for a long time, radiological features of pulmonary fibrosis similar to those found in idiopathic pulmonary fibrosis (IPF) are rarely observed. It is worth noting that new biological drugs, especially TNF- α antagonists (infliximab, adalimumab, etanercept) used in CD can lead to non-infectious complications including granulomatous disease and interstitial lung disease (ILD) with sub-acute or acute course resembling acute interstitial pneumonia (AIP). Occasionally, chronic eosinophilic pneumonia (CEP), OP, and diffuse alveolar haemorrhage (DAH) are also reported. The most frequently observed consequence of treatment with anti-TNF- α drugs, especially after using etanercept, is a mild granulomatous

reaction which usually lasts until the end of the biological therapy and does not require any additional medical intervention. The histopathological analysis shows pulmonary alveoli with a lymphohistiocytic infiltrate, the presence of non-necrotizing granulomas and fibrosing alveolitis, with negative results of microbiological culture. In these cases, the development of ILD has a more serious prognosis and is associated with a higher risk of death. Symptoms of ILD usually appear after three infusions of TNF-alpha antagonist, however it should be mentioned that in some studies a certain number of patients (10 out of 23) were additionally receiving modifying antirheumatic drugs (DMARDs), including methotrexate (MTX). An important element of diagnosis in the above-mentioned field is the identification of drug-induced ILD. Its differentiation from interstitial lung diseases caused by other factors is difficult to be performed basing on clinical, radiological and histological picture (Fig. 3). There is no linear relationship between the severity of symptoms and the dose of drugs. The first symptoms usually appear only after 5-7 days of latency. The following criteria are used to recognize the drug-induced ILD:

- a. Symptoms of ILD are not observed prior to initiating IBD treatment,
- b. The clinical picture of drug-induced ILD should correspond to that presented in literature reports concerning drug-induced reactions,
- c. Other diseases and other drugs should be excluded,
- d. Symptoms of drug-induced interstitial lung disease should resolve after discontinuation of a particular drug,
- e. Symptoms of drug-induced ILD recur, without any latent period, after continuing the treatment with the same drug [44, 55]. In some patients, eosinophilia in the colon tissue has been found to be higher in subjects with positive tests for oral allergens, which may indicate that atopy may be a common basis for the occurrence of pulmonary lesions in the course of IBD. In epidemiological studies, a high correlation between people suffering from chronic obstructive pulmonary disease (COPD) and those with IBD has been demonstrated. The risk of COPD development in patients with CD increases about 2.7 times, and 1.8 in patients with UC, as compared to healthy people [56].

In various studies, attention has been paid to the relationship between severity of IBD and lesions in the airways. In some of them, patients reported a greater number of respiratory symptoms during exacerbations of the disease course, and observed less symptoms when bowel disease subsided. An increased number of respiratory symptoms associated with a high endoscopic activity index value was indicated by patients with UC, however no correlation between the actual IBD activity and pulmonary lesions was detected. Changes in the pulmonary function test (PFT) were more numerous in patients with active IBD. Spirometry findings showed lower levels of FEV1, FVC, FEV1/VC in patients with IBD exacerbations and an elevated level of RV, RV/TLC between remissions. No relationship between the duration of IBD and the result of PFT as well as between

the results of the bronchial hyperresponsiveness test with the use of metacholine and the course of inflammatory bowel diseases was observed. Although some researchers have pointed to the possible correlation between the duration of IBD, and HRCT findings, no relationship was finally stated between them. The analysis of numerous clinical cases shows a higher number of respiratory complications with an increase in the duration of IBD exacerbations [57]. It should also be noted that although the systemic lesions in the respiratory system can occur in any age, the female gender is more predisposed. The most common symptom of airway inflammation is a cough with a profuse expectoration of purulent sputum. Lesions in the larynx and trachea can give hoarseness and stridor. Sometimes the bronchoscopic examination reveals the nodules of an inflammatory nature, which can to a large extent narrow the airways. If left untreated, they can lead to tracheal stenosis, bronchiectasis, deformities, and even fistulas. In these cases, clinical and histopathological picture presents granulomatous, necrotizing, purulent or obstructive bronchiolitis. Recent studies indicate an increased risk of asthma in UC and CD patients. The most common form of lesions, besides those in the airways and pleura, is the organizing pneumonia (OP) usually located in the upper regions of the lungs, but it can also involve the entire lungs and even lead to respiratory failure. Other types of ILD in the course of IBD are non-specific interstitial pneumonia (NSIP), desquamative interstitial pneumonia (DIP), and pulmonary interstitial emphysema (PIE). In BAL, a high percentage of lymphocytes can be found in CD patients and their characteristics may be identical to that of sarcoidosis. In differential diagnosis, it is useful to assess serum angiotensin-converting enzyme (ACE) activity (high in sarcoidosis, low in CD). Small, sterile, necrotizing nodules, which may undergo breakdown, have also been observed in the pulmonary interstitium of IBD patients. They are usually accompanied by necrotic bronchitis, which should be differentiated from granulomatosis with polyangitis (GPA) (Fig. 4) [55].

CONCLUSIONS

Evidence suggests that CD and UC, described as non-specific IBD characterized by varied symptoms in a number of systems, are not so much inflammatory conditions affecting the intestines, but rather a group of multisystem inflammatory diseases, in which all manifestations observed in other systems, such as the respiratory tract, are coexisting symptoms. The clinical symptoms presented by patients are manifested as an effect of the chronic inflammation representing the underlying cause of the condition. The key role in disease initiation is played by acute phase proteins such as IL-6, IL-12, IL-21, IL-23, TNF-alpha and INF-gamma; an important part is also played by the activation of membrane proteins such as CD40/CD40L and the general destabilization of immune system function, for example, inflammation of the mucous membranes. Additionally, immune system function is further disturbed

by the action of neuroendocrine peptides/amines. The pathomechanisms analyzed in this article manifest as different symptoms in various parts of the body, for example, where inflammation may be manifested by the presence of non-necrotizing granulomas in the respiratory tract. The granulomas occurring in the intestines show a number of histopathological and immunological similarities to those found in the airways. This similarity hints at the presence of an underlying connection between co-occurring diseases in different systems. It also highlights their destructive nature, manifested in the intestines as abscesses, ulcers, fistulas or post-inflammatory adhesions, and in the lungs as restriction resulting from pulmonary parenchymal disease, bronchitis, pleuritis and colobronchial fistulas; their occurrence is also associated with an increased risk of thromboembolism, coughing and dyspnea.

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Conflict of interest

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REVIEW ARTICLE

INTERNATIONAL LEGAL INSTRUMENTS IN THE FIELD OF BIOETHICS AND THEIR IMPACT ON PROTECTION OF HUMAN RIGHTS

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Introduction: A rapid development of biomedicine, genetics, pharmacology, transplantation and biotechnology has posed a number of problems to humanity, in particular, with regard to human rights protection in healthcare. These problems solution requires considering the achievements and propositions of biology, medicine, ethics and law. International legal standards in the field of bioethics are of significance in development of national states regulations on bioethics and biotic legislation.

Aim: To investigate the impact of international legal instruments in the field of bioethics on protection of human rights.

Materials and methods: In the research the international legal instruments and documents in the field of healthcare and bioethics were used. Civilizational, axiological, dialectical, systemic and comparative legal methods as well as systematization, analysis and synthesis were decisive in the research process.

Conclusions: Legal instruments in the field of biomedical technologies (directives and regulations) are mainly advisory by nature. In many cases, the problems arising in biotechnology are resolved through establishment and involvement of national supervision bodies: councils (commissions, committees) in bioethics as well as courts. An important role in protection of human rights in the field of biotechnology is played by the ECHR the decisions of which are dynamic, based on the Convention and consideration of national legislations. At the same time, a number of problems remain unresolved because of constant development of biomedical technologies, necessity to take into account the latest achievements and discoveries as well as all types and methods of applying of genetic engineering to humans. In general, insufficient attention is paid to the problems of medical biotechnologies application both at the international and national levels.

KEY WORDS: bioethics, biolaw, biomedicine, medical law, international medical law, international document

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INTRODUCTION

Rapid development of biomedicine, genetics, pharmacology, transplantation and biotechnology opens fundamentally new possibilities for improving the quality of life and its duration, but at the same time it may result in unpredictable and contradictory consequences. On the one hand, these possibilities include artificial insemination, surrogacy, transplantation, stem cell treatment, etc., but on the other – there are also the problems of human cloning, euthanasia, experiments on humans and animals. The complex and multidimensional nature of these problems requires attention of various sciences with their achievements and propositions, in particular, biology, medicine, ethics, law and psychology. It is also extremely important to have a common understanding of the international community problems, to establish the international legal principles and standards in the field of bioethics which must be the basis for national regulations.

It is quite natural that the issues that arise in genetics, pharmacology, transplantation and biotechnology are increasingly subject to legal regulation at the international level. The legal provisions enshrined in international regulatory and legal instruments with regard to bioethics

are becoming a part of national legislations and affecting protection of human rights.

THE AIM

The article aims to study the international legal instruments in the field of bioethics and their impact on protection of human rights.

MATERIALS AND METHODS

In order to achieve the goal a set of general and special scientific approaches as well as the methods of social and legal phenomena cognition were used, namely: civilizational and axiological methods – to study the value basis of bioethics; dialectical method – to identify the relationship and interaction of international, regional and national legislations in the field of healthcare and bioethics; systemic method – to investigate protection of human rights in the field of bioethics; comparative legal method – to analyze foreign experience of legal regulation and protection of human rights in bioethics. Their use in correlation and complementarity enabled us to achieve the goal of this

research, to provide a comprehensive understanding of the research subject, scientific reliability and credibility of researches' results.

RESULTS AND DISCUSSION

In modern science the issues related to the progress of genetics, genomics, pharmacology, transplantology, biotechnology and cloning mainstreamed not only scientific discussions within the relevant sciences but also caused a number of problems in understanding of legislation and legal regulation. This consequently resulted in the establishment of biolaw, biojurisprudence and biotic legislation. Scientists share the view that the object of bioethics is human intervention into the natural processes of treatment both environment and human nature. The bioethics subject is the related moral problems that arise – the problems of mankind survival and maintenance of life on the planet [1, p. 22]. Therefore, bioethics exists rather as an incessant field of ethical, religious, psychological and legal problems, constantly expanding and becoming more complicated and having no simple and unambiguous solutions, than as a scientific discipline with a clear subject and generally accepted common language. Within bioethics there are a lot of legal problems primarily related to promotion and protection of human rights in healthcare, the right to privacy, the right to information, etc.

In the 1990s at the intersection of philosophy of law, theory of law, constitutional, administrative, civil and international law on the one hand and medicine and bioethics – on the other, biojurisprudence started to take shape. The latter is considered by modern scientists as the newest scientific area aimed to maintain and legally protect human life in relation to an intensive development of biomedicine. The principle of the highest value of life of man as a biosocial being forms the basis of biojurisprudence.

The analysis of scientific sources shows that the term “biojurisprudence” was introduced into science by Roman Tokarczyk, a Polish scientist, Professor at Maria Curie-Skłodowska University in Lublin. The scholar presented his main propositions and key ideas in his writings “Biojurisprudence. Foundations of Law for the Twenty-First Century” (2008) [2] and “The Rights to Birth, Life and Death: Foundations of Biojurisprudence” (2012) [3]. The Tokarczyk concept of biojurisprudence grounds on the interpretation of the essence of understanding the deepest meaning of law – “life” (of human beings, society and other living beings) and in a generalized form is realized in the following propositions: life, primarily human life, determines the deepest meaning of the subject matter of jurisprudence and law; the current development of biological sciences (biotechnology) is based on artificial intervention in the natural processes of life; the trends in biotechnology, bioengineering and biomedicine indicate a gradual expansion of the scope of their artificial intervention in the natural processes of life; the consequences of artificial interference in natural processes of life are exposed to various appraisals, especially religious,

moral and legal ones; a variety of appraisals of artificial interference in natural processes of life is the basis for the conviction that not everything that is technically possible must be morally permissible; it is difficult to overestimate and even predict all the serious consequences of artificial interference in natural processes of life [3]. The above ideas and propositions were further developed by M. Weiss, K. Wolska-Lipiec, R. Tokarczyk (Poland), M. Kashintseva, O. Merezhka, V. Plavich, P. Rabinovich, R. Stefanchuk, S. Stetsenko, M. Medvedeva (Ukraine), V. Kruss, M. Maleina, M. Matuzova, G. Romanovsky, V. Salnikov (Russia), etc.

Biomedical achievements and discoveries are the major factor which constantly encourages scientific research of philosophical, legal, general theoretical and sectoral problems in the field of bioethics. Recognition of the biotechnology and biomedicine achievements at the international level has intensified the need for legal regulation of public relations in healthcare and bioethics as well as establishment of biotic legislation and biojurisprudence.

Despite the fact that the term “biojurisprudence” appeared in the 1990s, the problems in this area and attempts to resolve them legally have a much longer history. The establishment of international legal instruments which significantly affect protection of human rights in the field of bioethics has lasted for more than 70 years since 1946. For the first time, in the Constitution of the World Health Organization it was declared that “The enjoyment of the highest attainable standard of health is one of the fundamental rights of human being without distinction of race, religion, political belief, economic or social condition”. The health of all peoples is a fundamental factor in achieving peace and security and depends on the closest cooperation of individuals and states. The achievement of any state in improvement of health and health care is valuable for everyone [4]. These and other provisions of the WHO Constitution laid the foundation for international medical law, national law and legislation in the field of bioethics of most developed states.

The history of the legal instrument's establishment in the field of bioethics shows that the most high-profile debate on the bioethics problems by the international community first took place in 1946-1947 in Nuremberg. It was dedicated to the medical human subject research conducted during World War II, namely the forced medical research, murders of war prisoners for the August Hirt anatomical collection, forced euthanasia, forced sterilization, etc. One of the consequences of this process was the creation of the Nuremberg Code, which regulated the conduct of scientific experiments and human subject research as well as introduction of ethical standards for scientists engaged in medical experiments. The Code said that for conduction of human subject research the voluntary consent of the human subject is essential. This voluntary consent should be based on full awareness of the human subject after providing him with full information about the nature, duration, purpose of the experiment, methods and means of its realization, all alleged inconveniences and dangers of the experiment, possible consequences for physical and

mental health of the subject, which may arise as a result of his participation in the experiment. Even though the requirements of this Code only became moral imperatives and were not binding they were then embodied in a number of national legislations in the field of medical research on human subjects. In addition, the Code encouraged the mankind and individual states to obtain scientific knowledge about the maintenance of human health.

During 20th century a row of international legal instruments of an integrated nature were adopted at the international level, which along with the right to life and health laid the basis for settlement of biotic issues (the Universal Declaration of Human Rights adopted in 1948, the International Covenant on Economic, Social and Cultural Rights adopted in 1966). Among the three groups of international medical documents distinguished in modern science [5], namely: 1) acts and documents concerning the issues of the healthcare sector reform (the Declaration of Alma-Ata, the Ljubljana Charter, etc.); 2) acts and documents, which provide for the legal status of participants in healthcare relationship (the Declaration of Lisbon on the Rights of the Patient, the European Charter of Patients' Rights, etc.), the international legal instruments, which lay the grounds in the field of bioethics, should be referred to group 3, that is, the acts and documents with regard to specialized issues in medicine and healthcare (the Declaration on Euthanasia, the Declaration of Sydney on human death, the Principles for the Protection of Persons with Mental Illness and the Improvement of Mental Health Care, the Universal Declaration on the Human Genome and Human Rights, etc.).

Among the international instruments in bioethics which provide for the ethical standards and conduct of scientific research on humans and are based on the principle of personal autonomy special attention should be paid to the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects [6]. The document was adopted by the 18th WMA General Assembly held in Helsinki (Finland), in July 1964. Subsequently, the document was repeatedly revised and amended, namely: at the 29th WMA General Assembly, Tokyo (Japan), October 1975; the 35th WMA General Assembly, Venice (Italy), October 1983; the 41st WMA General Assembly, Hong Kong, September 1989; the 48th WMA General Assembly, Somerset West (South Africa), October 1996; the 52nd WMA General Assembly, Edinburgh (Scotland), October 2000; the 53rd WMA General Assembly, Washington, DC (the USA), 2002 (explanatory note to §29); the 55th WMA General Assembly, Tokyo (Japan), 2004 (explanatory note to §30); the 59th WMA General Assembly, Seoul (South Korea), October 2008.

The establishment of biolaw and biomedical legislation was influenced by a number of international instruments adopted by various entities, primarily the United Nations (UN), the World Medical Association (WMA), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the Council of Europe (COE), the Parliamentary Assembly of the Council of Europe (PACE), the Committee of Ministers of the Council of Europe, the European

Parliament (EP). In addition, the bioethical principles and standards are contained in the bioethical international, regional and national legal documents of specialized international and regional integration organizations, in the national legislations of different countries.

In terms of rule-making the most important among them are the following: the UN International Convention on the Elimination of All Forms of Racial Discrimination of 21 December 1965, the UN Convention on the Elimination of All Forms of Discrimination Against Women of 18 December 1979, the UN Convention on the Rights of the Child of 20 November 1989, the UN Convention on Biological Diversity of 5 June 1992, the UN *Standard Rules on the Equalization of Opportunities for Persons with Disabilities* of 20 December 1993). In addition, a number of international instruments with regard to bioethics issues were adopted by the UN specialized agencies, in particular, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

The World Medical Association (WMA) – the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects adopted in 1964 and amended in 1975, 1989, 1996 and 2000; the Declaration of Lisbon on the Rights of the Patient adopted in 1981 [7].

The UNESCO [8] – The UNESCO Recommendation on the Status of Scientific Researchers of 20 November 1974, the UNESCO Declaration on Race and Racial Prejudice of 27 November 1978, the Universal Declaration on Bioethics and Human Rights of 1978. The UNESCO Bioethics Programme was initiated in 1993, with the creation of the International Bioethics Committee (IBC), which is the first and only Bioethics Committee with the global scope of activities and expert membership. The UNESCO programme is tasked to reveal the most significant bioethics issues for different regions of the world in order to identify and implement appropriate strategies that would promote reflection on ethical and legal issues at the regional and sub regional levels, as well as to strengthen national capacities and international cooperation in the field of bioethics [9]. Subsequently, the UNESCO Declaration on the Responsibility of the Present Generations Towards Future Generations of 12 November 1997, the UNESCO Universal Declaration on the Human Genome and Human Rights of 11 December 1997, the UNESCO Universal Declaration on Cultural Diversity of 2 November 2001 were adopted. In 2005, the UNESCO International Bioethics Committee has developed the Universal Declaration on Bioethics and Human Rights [10].

The Council of Europe (COE) – The Convention for the Protection of Human Rights and Dignity of the Human Being regarding the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, adopted by the Council of Europe in 1997 (entered into force in 1999), and Additional Protocols thereto [11]. The Convention sets out the fundamental principles regarding patients' rights, namely equitable access to medical care, protection of the right to informed consent, confidentiality and the

right to acquire information. These principles are binding for the states that've ratified the Convention.

The Parliamentary Assembly of the Council of Europe (PACE) – Recommendation 934 (1982) on Genetic Engineering, Recommendation 1046 (1986) on the Use of Human Embryos and Foetuses for Diagnostic, Therapeutic, Scientific, Industrial and Commercial Purposes, Recommendation 1100 (1989) on the Use of Human Embryos and Foetuses in Scientific Research, the Resolution on Cloning (1998), the *Directive 98/44/EC on Legal Protection of Biotechnological Inventions* (1998), etc. [12].

The Committee of Ministers of the Council of Europe – the Resolution (78) 29 on Harmonisation of Legislations of Member States Relating to Removal, Grafting and Transplantation of Human Substances (1978), the Recommendation No. 92 on the Use of DNA Analysis in the Criminal Justice System (1992), the Convention on Human Rights and Biomedicine (European Convention on Bioethics) (1996) [13].

The European Parliament – the Resolution on Artificial Insemination “in vivo” and “in vitro” (1989), the Resolution on Ethical and Legal Problems of Genetic Engineering (1989). [14]

The activities of the Council for International Organizations of Medical Sciences (CIOMS) also deserve our consideration. The CIOMS has adopted a number of instruments – the International Ethical Guidelines for Health-related Research Involving Human Subject of 1982, amended in 1993 and 2002. In addition, in 2002 Active Citizenship Network in collaboration with public organizations from different EU countries developed the European Charter of Patients' Rights [15] which clearly and fully interpreted the rights of patients.

Among the ideas and provisions of the specified legal instruments in the context of human rights protection there are the provisions of special significance which provide for protection of dignity and identity of the person, guarantee everyone, without discrimination, respect for their integrity and other rights and fundamental freedoms with regard to application of biology and medicine, the prevalence of human being interests over sole interests of society or science. The provisions also set out the need to comply with relevant professional obligations and standards for any medical intervention, including research; the need for a person's voluntary informed consent to medical intervention; protection and respect for private and family life in collection, transmission and storage of personal medical data; prohibition of discrimination against a person on the basis of his genetic heritage; prohibition of commercial use of the human body. At the same time, it should be noted that the above instruments largely reflected the intensive theoretical research of bioethical problems of scientific experiments, and unfortunately, they were not legally binding.

The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine can be considered the first international binding instrument in the field of bioethics. The Convention was adopted by the Council of Europe in

Oviedo, on 4 April 1997 and entered into force in 1999. Subsequently, the Convention was supplemented by Additional protocols as of 12 January 1998, 24 January 2002 and 25 January 2005 [16].

The activities of the European Court of Human Rights have a significant impact on the development of bioethics, national and international legislation in the field of health protection and biotic issues. The analysis of the ECHR practices [17] gives the grounds to identify several groups of human rights, in relation to which the issues of bioethics were raised, namely:

- 1) reproductive rights, prenatal diagnosis [Draon v. France, 2006] [18], the right to a legal abortion [Tysiacy v. Poland, 2007] [19]; [R. R. v. Poland, 2011] [20], [Z v. Poland, 2013] [21], [Dubská and Krejzová v. the Czech Republic, 2014] [22], [Kosaitė-Čypienė and others v. Lithuania, 2019] [23];
- 2) artificial insemination [Evans v. the United Kingdom, 2007] [24], [Dickson v. the United Kingdom, 2007] [25], [S.H and Others v. Austria, 2011] [26], [Costa and Pavan v. Italy, 2013] [27], [Parrillo v. Italy, 2015] [28], [Foulon and Bouvet v. France, 2016] [29];
- 3) euthanasia [Pretty v. the United Kingdom, 2002] [30], [Haas v. Switzerland, 2011] [31], [Jack Nicklinson v. the United Kingdom and Paul Lamb v. the United Kingdom, 2015] [32], [Koch v. Germany, 2012] [33], [Gross v. Switzerland, 2014] [34];
- 4) the patient's consent to medical examination or treatment: a) general issues regarding consent [Hoffmann v. Austria, 1993] [35], [Arskay v. Ukraine, 2014] [36], [Petrov v. Latvia, 2014] [37], [M.S. v. Croatia (no. 2), 2015] [38], [Lambert and others v. France, 2015] [39], [Bataliny v. Russia, 2015] [40], [Fernandes de Oliveira v. Portugal, 2019] [41]; b) consent to pelvic examination [Juhnke v. Turkey, 2008] [42], [Salmanoğlu and Polattaş v. Turkey, 2009] [43], [Konovalova v. Russia, 2014] [44]; c) consent to sterilization [V.C v. Slovakia, 2011] [45], [Y.Y. v. Turkey, 2015] [46], [S.V. v. Italy, 2018] [47], [Bogdanov v. Russia, 2015] [48];
- 5) ethical issues concerning HIV and other life-threatening diseases: a) threat of eviction [Francisco J. Arcila Hena v. the Netherlands, 2003] [49], [N. v. the United Kingdom, 2008] [50], [Kiyutin v. Russia, 2011] [51]; b) isolation of patients [Enhorn v. Sweden, 2005] [52]; c) confidentiality [Colak and Tsakiridis v. Germany, 2009] [53], [I.B. v. Greece, 2013] [54], [Y. v. Turkey, 2015] [55]; d) preventive measures / access to treatment [Claes v. Belgium, 2013] [56], [Savinov v. Ukraine, 2015] [57], [Catalin Eugen Micu v. Romania, 2016] [58], [Karpylenko v. Ukraine, 2016] [59], [Blokhin v. Russia, 2016] [60];
- 6) storage of fingerprints, cell samples and/or DNA by authorities: [Gillberg v. Sweden, 2012] [61], [M.K. v. France, 2013] [62], [Antonio Peruzzo v. Germany and Uwe Martens v. Germany, 2013] [63], [Elbert v. Latvia, 2015] [64];
- 7) the right to access information on biological origin: [Andrzej Kłoczek v. Poland, 2012] [65], [Grönmark v. Finland, 2010] [66], [Krušković v. Croatia, 2011] [67], [Kautzor v. Germany, 2012] [68], [Ahrenz v. Germany, 2012] [69].

In its decisions the ECHR repeatedly refers to international instruments in the field of bioethics including the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine adopted in Oviedo on 4 April 1997, in particular, in the following cases: [Cyprus v. Turkey, 2001] [70], [Vo v. France, 2004] [71], [Evans v. the United Kingdom, 2007] [24], (the complaint was declared inadmissible, article 5 of the Oviedo Convention was cited); [Costa and Pavan v. Italy, 2013] [27], [Lambert and others v. France, 2015] [39], [Bataliny v. Russia, 2015] [40]. In addition, the ECHR considers the activities of the Council of Europe in this area, in particular in the following cases: [Parrillo v. Italy, 2015] [28], [Paradiso and Campanelli v. Italy, 2015] [72].

Special attention in the context of human rights protection should be paid to the issues of transplantation of human organs and tissues [73] and the use of reproductive technologies [74].

The analysis of the ECHR practices shows that most often the court deals with the issues of reproductive rights, artificial insemination, consent of the patient to medical examination and treatment, the right to access information on the biological origin, euthanasia, ethical aspects in relation to HIV, storage of biological material by public authorities. Such cases primarily fall within the scope of Article 8, as well as Articles 2, 3, 5, 6. With the further rapid development of biotechnology, the applications regarding gene therapy, stem cell research and treatment as well as cloning will be the subjects of consideration. The ECHR draws attention to a considerable discretion of states in a number of issues (in particular, the issue of adequate and timely medical care in the framework of prenatal research (A.K. v. Latvia) [75]; the need to maintain a fair balance between the interests of society on the one hand, and protection of the right to respect privacy – on the other (Draon v. France, 2006) [18]. The European Court of Justice has repeatedly noted that the concept of “inviolability” is unclear, especially when positive obligations of the state depending on the circumstances are concerned. Therefore, in assessing the positive obligations of the state the need to respect the rule of law – a fundamental principle of democratic state is crucial) (Carbonara and Ventura v. Italy, 2000) [76]. Respect for the rule of law requires that national law provide legal protection against public authorities’ interference in the rights guaranteed by the Convention (Hasan and Chaush v. Bulgaria, 2000) [77]. Special attention should be paid to the fact that the ECHR emphasizes that the Convention is aimed at ensuring rather real and valid rights than theoretical and illusory ones (Airey v. Ireland, 1979) [78].

CONCLUSIONS

Summarizing the above stated, we should note that at the international level there are many international legal instruments of an integrated nature, which, along with the human right to life and health, lay the basis for settlement of biotic issues. At the same time, especially in the field of biotechnology most legal instruments are of a regional character. The main law-makers in this area are the UNESCO, WHO, COE and EU. The legal instruments in the field of biomedical technologies (directives and regulations) are mainly advisory by nature. Directive 98/44

concerning legal protection of biotechnological inventions is an exception. Comparing the experience of legal regulation in the field of biotechnology in foreign countries we can conclude that there are significant differences in the regulation of medical biotechnology, existence of general, abstract and inaccurate provisions. Italy has the most stringent legislation (which prohibits all ethically unacceptable types of medical biotechnology); the most liberal legislation is that of the UK (it provides for permission for therapeutic cloning and the use of embryonic stem cells from any source). Therapeutic cloning is also legally permitted in Belgium, Denmark and Switzerland. In many cases the problems arising in the field of biotechnology are resolved through the establishment and activities of national supervision bodies: councils (commissions, committees) on bioethics, in courts. An important role in protection of human rights in the field of biotechnology is played by the ECHR, with the decisions being dynamic, based on the Convention and, at the same time, taking into account national legislations. At the same time, some problems remain unresolved because of the constant development of biomedical technologies, the need to take into account the latest achievements and discoveries, all types and methods of applying genetic engineering to humans. In general, insufficient attention is paid to the problems of medical biotechnologies application both at the international and national levels.

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REVIEW ARTICLE

THE SCIENTIFIC AND PEDAGOGICAL HERITAGE OF DOCTOR N.I. PIROGOV

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ABSTRACT

The aim: was to analyze the scientific and pedagogical heritage of N.I. Pirogov, which remains relevant in the modern medical and pedagogical community, and opens new little-known pages of the scientific, pedagogical and medical activity of the doctor.

Materials and methods: The authors conducted a thorough analysis of scientific publications by N.I. Pirogov and papers devoted to his activity as a doctor, educator and researcher using analytical, comparative and bibliosemantic methods.

Conclusions: The study suggested the new chronological boundaries in the scientific and educational heritage of N.I. Pirogov. The developed periodization accurately reflects the beginning of his scientific and pedagogical path in medical education, specifically refers to the period of his work in Germany as a supervisor of professorial candidates from 1862 to 1866, and the period of his participation in Sevastopol (Crimean) campaign and in the Franco-Prussian War, which was very important for the development of military field surgery.

KEY WORDS: N.I. Pirogov, higher education, scientific and pedagogical heritage, education and training of future doctors

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INTRODUCTION

The reform of national healthcare system has identified priorities for the competence paradigm of training future doctors and updating educational programs for healthcare professionals. The combination of modern developments in medical science with major cultural achievements and the vast heritage of prominent Ukrainian doctors should extrapolate the problem of updating Ukrainian medical education at all levels of professional training and preserving its national identity. In our opinion, the appeal to the scientific and pedagogical heritage of Nikolai Ivanovich Pirogov (1810 – 1881) – a prominent scientist, founder of topographic anatomy and military field surgery, doctor, educator, humanist, enlightener and reformer of national education, – is a basis for generating new ideas and developments in the field of medical education, as a classic example of a combination of professional and intellectual, theoretical and practical, social and personal aspects.

THE AIM

The aim of the research is to analyze the scientific and pedagogical heritage of N.I. Pirogov, which remains relevant in the modern medical and pedagogical community, and opens new little-known pages of the scientific, pedagogical and medical activity of the doctor, giving impetus for a systematic analysis of his life-work.

MATERIALS AND METHODS

The authors conducted a thorough analysis of scientific publications by N.I. Pirogov and papers devoted to his activity as a doctor, educator and researcher using analytical, comparative and bibliosemantic methods. The study of scientific and pedagogical literature showed that N.I. Pirogov's personality was researched by scientists of different fields, but despite a considerable amount of research of pedagogical views, N.I. Pirogov's scientific and pedagogical heritage needs further systematization and special consideration. Most modern scholars (O. Bieliaieva, L. Bondar, N. Zarems'ka, M. Levkivskyi, O. Mykytiuk, O. Pisots'ka, N. Pobirchenko, S. Sysoieva, I. Sokolova et al.) encourage to study the pedagogical heritage of N.I. Pirogov due to importance and adaptability of pedagogical developments of the scientist for the present pedagogical activity for the school system of national education under conditions of its reformation.

The scientists' appeal to educational ideas of N.I. Pirogov has been going on for over a hundred years, and has a great deal of proficiency in the form of profound dissertation studies of his works and pedagogical activity, considering the humanistic nature of his pedagogy (O. Horchakova, K. Fedoruk et al.) and views on the upbringing of the young person's individuality (O. Bakushkina, O. Dolia, V. Inozemtsev, O. Savytska et al.). Some works are devoted to research and teaching activities of N.I. Pirogov in higher

medical education and his medical and educational ideas of improving the efficiency in medical practice (O. Bieli-aieva, M. Voronov, I. Haivoronskyi, O. Klantsa, M. Kushyk, B. Pazynych, A. Postoi, H. Semanov, O. Okulov, E. Khodosh and others).

The scientific and medical heritage of N.I. Pirogov has been extrapolated by modern scientists to solve problems of anatomy, military medicine, forensic medicine and other medical branches.

REVIEW AND DISCUSSION

In our opinion, the analysis of N.I. Pirogov's scientific and pedagogical heritage deserves particular attention from the standpoint of the current state of national medical education and the importance of his scientific work for its reformation in terms of entering the European and world educational space. In the scientific literature, there are several periods in the formation of N.I. Pirogov's pedagogical views. For example, O. Horchakova in the study of the formation and development of the humanistic pedagogical concept of N.I. Pirogov proposes 5 periods, "corresponding to the stages of his pedagogical activity" [1, p. 6-7]: the first period (1810-1828) is the stage of formation of the character, the worldview of the scientist, his definition in scientific interests and priorities of research in the medical field; the second period (1828-1841) is the stage of scientific and medical search and experience of teaching practices in Tartu University; the third period (1841-1856) is the stage of formation of the pedagogical concept of the scientist at the Medical and Surgical Academy in St. Petersburg and publication of the article "Issues of Life"; the fourth period (1856-1861) is the stage of practical realization of the pedagogical concept of Pirogov in Odesa and Kyiv educational districts; the fifth period (1861-1881) is the time of recognition of pedagogical views of Pirogov in the society and dissemination of his ideas in the pedagogical theory and practice. In our opinion, the chronological boundaries of periodization, which have been outlined, do not accurately reflect the beginning of his scientific and pedagogical path in medical education, they do not specifically mention the period of his work in Germany as a supervisor of professorial candidates from 1862 to 1866, and the period of his participation in Sevastopol (Crimean) campaign and in the Franco-Prussian War, which was very important for the development of field surgery. These "white spots" in the periodization of research and educational views of N.I. Pirogov need appropriate correction on the basis of study of new sources and scientific publications in medical and pedagogical heritage of the famous doctor.

The analysis of scientific works by N.I. Pirogov and publications devoted to his activity as a doctor, scientist and educator, demonstrate that the first steps in the formation of scientific and pedagogical views of N.I. Pirogov were made during his studies from 1824 to 1828 at the Medical Faculty of Moscow University. Under the influence of lectures delivered by M.Ya. Mudrov (1776-1831) – a professor, clinician and brilliant educator at the university, the young

N.I. Pirogov became interested in pathological anatomy, although it was not taught as a separate subject at that time. In the monograph by the famous pathologist, professor M.K. Dal, it is noted that according to the statement of N.I. Pirogov, the lectures by M.Ya. Mudrov "continually explained the need to study pathological anatomy and dissection of corpses... and thereby made me want to get acquainted with this *terra incognita*", and later, as M.K. Dal wrote, N.I. Pirogov said that university studies "left a deep impression on the whole life, slammed into the soul and gave a well-known direction for life" [2, p. 4-5].

The next period of formation of N.I. Pirogov as the scientist was the so-called "Dörpt period". This is when N.I. Pirogov was sent to Dörpt University in Germany after completing his studies at the University to write a doctoral thesis. The topic of dissertation was suggested by N.I. Pirogov, and it was entitled: "Can Ligation of the Abdominal Aorta Give a Favorable Result in the Inguinal Aneurysms?". In the content of his dissertation, N.I. Pirogov used the elements of pathological anatomy, thereby establishing the technical possibility of ligation of the abdominal aorta. He suggested the most convenient location for such ligation and identified operative approaches to the abdominal part of the aorta. In his dissertation, on the basis of animal studies, N.I. Pirogov was the first to demonstrate the presence of collateral circulation and its connection with the circulation in the abdominal aorta and spinal cord. The thesis was defended on August 31, 1832. The text of the thesis in 40 pages was published in Latin and translated into Russian for publication in *Bulletin of Natural Sciences and Medicine* that was monthly published in Moscow and was edited by Dr. Iovskiy [2, p. 4-5].

A few years later, in Berlin, N.I. Pirogov showed his dissertation to the famous surgeon Opitz, who was amazed by the practical achievements of the young surgeon and ordered to translate it into German as a matter of urgency [3, p.11]. Thus, in 1838, it was published in a German surgical journal. The stay of N.I. Pirogov in Dörpt was not limited to writing and successful defending of his thesis. The first scientific work by N.I. Pirogov was published in 1829 and was dedicated to anatomic and topographic description of the hip and groin area. At that time, N.I. Pirogov conducted numerous practical researches in the field of pathological anatomy; he worked with German professors and surgeons Schlemme, Langenbeck and Textor, whose experience N.I. Pirogov highly praised and criticized at the same time. For example, N.I. Pirogov had some practical remarks to certain techniques and surgical instruments of Langenbeck, though he highly appreciated his operational skills. Professor Textor received the most severe criticism from N.I. Pirogov, because of the slowness of his operations, which the young scientist considered unacceptable. At the same time, the young N.I. Pirogov greatly appreciated Schlemme's operating experience and found many common scientific interests in anatomy [3, p. 11-12]. Through his experimental studies, the young doctor aspired to change the attitude of well-known surgeons to pathological anatomy and to gain recognition of his

active practices in this field. His anatomical experiments were supported by the famous German professor Moyer, who supported the young N.I. Pirogov in the direction of development of pathological anatomy. Moyer gave him the opportunity for extensive surgical practice in his clinic and facilitated his research activities. N.I. Pirogov's work at Dörpt Surgery Clinic demonstrated his undeniable talent as a scientist and surgeon, therefore his assignment as a Professor of Surgery at Dörpt University in 1836 was a natural result of his extensive work in surgery and anatomy.

The beginning of N.I. Pirogov's professorship and teaching activities was associated with publication of the two volumes of "Annals of Surgical Clinic", which were the first attempt to share with young doctors the experience in surgery and to predict their errors in practice [2, p. 9]. The critical attitude of Pirogov to his medical activities in this scientific publication was a very unusual recognition of one's own mistakes by the professor and practicing surgeon. The world appraisal of Pirogov as a leading scholar of the time in the field of operative surgery were heralded by the work "Surgical Anatomy of Arterial Trunks and Fascias", which was published in Dörpt in 1837 in Latin, German and French. The work was primarily written for young surgeons in order to show the relationship between pathological anatomy and surgery in their practical dimension, and to teach them new techniques for providing surgical care [2, p. 10]. For this work, N.I. Pirogov later received the Demidov Prize of the Academy of Sciences.

Lectures of the young professor N.I. Pirogov were very different from the ordinary lectures of professors at Dörpt University. He was the first to introduce simultaneous teaching of theoretical and practical surgery with assigning of students to patients at clinics. Researchers in the scientific and pedagogical heritage are unanimous in that N.I. Pirogov had an extraordinary lecturing skill in delivering meaningful, sophisticated material, which was accompanied by his own hands-on research and prompted students for scientific inquiry and enhancing their professional competence. One of the priority teaching tasks of N.I. Pirogov was the development of students' scientific thinking and motivation to learn from other people's mistakes. This was brilliantly demonstrated to his students during his lectures. The peculiarity of his lectures was that in actual clinical cases he pointed the necessity of knowledge in surgical anatomy of that body part, which needed surgery. Such a clinical and pathological analysis, according to N.I. Pirogov, enabled a doctor to better perform medical diagnostics and prevent errors in surgery. The desire to change the teaching and learning methods was not positively favored by the German education system and, in our opinion, was the reason for N.I. Pirogov's return to St. Petersburg in 1840, where he was invited to head the Department of Surgery at the Academy. However, due to bureaucratic obstacles of the time N.I. Pirogov was officially appointed to the position of Professor at the Academy only a year later, in 1841. It was in 1841 that a new period of teaching activity of Pirogov at St. Petersburg Medical and Surgical Academy began, as well as the continuation of

his practical surgical activity as the Head of the Surgical Department of the Second Military Land Hospital [3, p.18]. As an educator, N.I. Pirogov considered it necessary to constantly combine lectures and practical classes. He paid attention to the use of his own research, clinical experience, emotional connection with the audience and the use of clarity in teaching. In 1846-1852, he taught students of the 5th year of study in pathological anatomy at the sectional table, delivered lectures "On Typhoid Lesions and Typhoid Deposits in the Lung, Spleen, Kidneys and Intestines", "On sepsis after Typhoid", "On Typhoid Process" [5, p. 9-10], as well as presented reports in medical communities on the features of manifestation of typhoid fever and on his own pathological and anatomical studies of patients who died. N.I. Pirogov continuously used the knowledge and experience of well-known foreign doctors Pettenkofer, Seitz, Louis and Rokitskyi in the fight against typhus epidemic during his lectures and in his professional medical practice during an epidemic in 1848.

During this period, N.I. Pirogov actively participated in combat events in the Caucasus region (1847) as a military surgeon of the acting army, and participated in the defense of Sevastopol (1854-1855) as a surgeon and organizer of military medical service. In particular, during Sevastopol (Crimean) campaign, he organized a special epidemiological and quarantine hospital (more than 1000 beds) that were located along the military deployment that made it possible to timely prevent the spread of epidemic in the army [5, p. 11]. During his work at the Medical and Surgical Academy, N.I. Pirogov developed a general academic prosectorium and pathoanatomical museum of the Academy, managed the Anatomical Institute (approved by his project) [6, p. 56], and for his scientific achievements in the medical field he was elected as a corresponding member of the Russian Academy of Sciences of the Biological Branch and academician of the Medical and Surgical Academy.

In our opinion, professorship at the Academy and regular meetings with students formed N.I. Pirogov's own vision of the development of education, pedagogy and personal upbringing of the young man's personality, therefore his appointment as a trustee of Odesa educational district in 1856, and a trustee of Kyiv educational district since 1858 after his retirement from the Medical and Surgical Academy, was a natural development of his scientific and pedagogical activity of a scientist and his desire to influence education of the young generation. He noted that "in pedagogical activity I was mostly concerned about the agreement between school and life, about the freedom of scientific research, about the excitement of respect for human dignity and truth in students and teachers" [7, p. 937-938].

In our opinion, the work by A. Rozhdestvina "Mykola Ivanovych Pyrohov as a Teacher" (1902) is a deep and original research of this period in his scientific and pedagogical activity. In this work, A. Rozhdestvina detaches from Pirogov's characteristics as a teacher, administrator and devotes her research to Pirogov as a theorist, teacher, drawing attention "to the ideas of the great teacher, which

were most important both in principle and practical respects" [8, p. 7]. The book reveals and organizes scientific and pedagogical views of N.I. Pirogov on the following issues: education and training; the role of sciences in the educational process; the importance of visualization and words in learning; the teaching methods and their peculiarities; the importance of a foreign language for education of young children; the duty of the society in relation to education and training. Pedagogical ideas of N.I. Pirogov on the formation of culture of mental work in young scientists are further analyzed and described in a very original way by I.O. Sikorskyi in his work "N.I. Pirogov as a Thinker and a Man" (1910). A well-known psychiatrist, professor at Kiev University of St. Vladimir, he notes that N.I. Pirogov, "like Shakespeare," makes a clear dependence in shaping the personality of a young man of "university age" from their systematic pursuits of science and mental work. Interest in scientific activity creates "physiological conductivity in the mechanism of neuro-psychic innervations" in a young man and is a "psycho-hygienic tool" for the development of self-control, nurturing freedom, formation of vital, moral principles of being. N.I. Pirogov recommended that young people should spend their youth years as he did in education and scientific activity. It is this persistence to study the sciences at a young age that ensures success and protects young people from "wandering and disturbance" [9, p. 9-10].

During this period, excited with reforming the educational system, N.I. Pirogov received great support and appraisal from the progressive professors of the time, teachers and students. Perhaps the most striking characteristic of the scientist in the position of a trustee of Odesa and Kyiv educational districts is the feedback on his work after his retirement in 1861 due to the state of health. In fact, various Kyiv and Odesa educational institutions organized a solemn ceremony at which N.I. Pirogov encouraged students to stay true to science [10, p. 908-910]. In memory of his teaching at Kiev University, N.I. Pirogov presented students with his portrait, which is now kept in one of the university auditoriums as a symbol of progressive ideas and reforms, which were the main tenets of the scientist's activity as a trustee. After his retirement, the scientific and pedagogical activity of N.I. Pirogov continued in Germany as the head of the professorial candidates, who were sent to continue scientific work under his tutelage [11, p. 37]. Scientific knowledge, experience in Germany, personal relations and organizational abilities of N.I. Pirogov over the period of four years helped more than one hundred professorial candidates in different specialties to choose scientific supervisors among the leading European scientists, to study the current directions of research of that time and to develop their own scientific topics and programs that could be further developed at national scientific universities. This period of the scientist's life involved the possibility of implementing his scientific experience, his knowledge in organization of research work of young scientists. For Pirogov, organization of research by professorial candidates at the European universities was commonly related with

practice, accompanied by the creation of favorable conditions for classes, sufficient material support of researchers abroad and ensuring the introduction of scientific developments in the domestic science after returning home. N.I. Pirogov was very attentive to professorial candidates, highly valued their scientific abilities and hard work, and recommended his students to the departments of leading universities.

In Heidelberg, and then in Berlin, N.I. Pirogov organized literary evenings at his home, which the youth called Pirogov's Saturdays. Professor I. Sokolovskyi says that these Saturdays raised topical issues of life and science, and, typically, there were ardent discussions and debates in which Pirogov was active, but "willingly conceded the word to each of those present" and "was strong in dialectics and found particular pleasure in scientific discourse" [12]. As a teacher and reformer in Germany, N.I. Pirogov continues to analyze the problems of higher education and writes a series of "Letters from Heidelberg", as well as the article "The University Issue", published as a separate brochure, in which he offers his competent vision of university reform with qualitative changes in "material status" of the university life, "division of rank and position", "free competition", "development of respect for the fact" [13, p. 324-393]. However, the most significant scientific work by Pirogov in this period was the publication of "The Beginning of Field Surgery" in German. The work was published in Leipzig and became an indispensable guide for surgeons in the organization of hospitals, medical administration, dressing rooms and antiseptic wound treatment. N.I. Pirogov briefly described his experience of military and medical work in twenty paragraphs of the book, which were then published in 2 volumes in Russian.

N.I. Pirogov witnessed the implementation of his recommendations on organization of hospital surgery a few years later during the Franco-Prussian war as a representative of the Society of Care for Wounded and Sick Soldiers. Within 5 weeks, N.I. Pirogov inspected 70 military hospitals and noted that all foreign doctors had comprehensively applied his methods of treatment and organization of medical care for the wounded and sick. As a result of this trip in 1871, he published "The Report on the Visit to Military Health Facilities in Germany, Lorraine and Alsace in 1870", and after a period of six months in the Russo-Turkish War, N.I. Pirogov wrote "Military and Medical Treatment and Private Care at the War Theater of Bulgaria and in the Rear of the Active Army" in 1879. The importance of these books for the formation and development of field surgery was so crucial that many researchers refer to them as the real scientific feat of N.I. Pirogov [11, p. 57-63].

The active period of scientific and pedagogical practice of N.I. Pirogov continued in 1879-1881 at Vyshnia estate near Vinnytsia. At this time, he wrote the memoir "Issues of Life. The Diary of an Old Doctor". This work became the last one in Pirogov's heritage on the issues of human education. The humanistic orientation of Pirogov's entire life was reflected in his work as observance of the norms of Christianity, civic duty, ethics and morality. Researchers

note the anthropological approach of the scientist to the essence of a man [14; 15]. Solving problems of education and training, which are strongly linked, were considered by N.I. Pirogov as crucial in the formation of personality, his/her spiritual foundations, ideas about the world, values and way of being. Until the last days of his life, the severely ill N.I. Pirogov worked on the manuscript of his "diary". These memoirs remain relevant both to modern pedagogical science and to the formation of a modern personality of the 21st century.

Thus, a deep analysis of various sources gives us the opportunity to develop a new periodization in scientific and pedagogical activity of N.I. Pirogov, namely:

The 1st period: formation of a scientific (1824-1828) – study at the Medical Faculty of Moscow University, where under the influence of prominent professors of that time the young Pirogov developed a scientific interest in complex medical processes and problems, as well as the desire to solve them with new non-standard methods.

The 2nd period: Dörpt (1828-1832) – training at the professorial institute of Dörpt University, writing the first scientific work "What is Observed during Operations for Ligation of Large Arteries" (1829), defense of doctoral thesis, dedicated to the ligation of the abdominal aorta (1832).

The 3rd period: teaching (1833-1839) – scientific and practical work in the clinics of Germany (1833-1835) and France (1838), the beginning of teaching activities at Dörpt University (1836), publication of "Annals of the Surgical Clinic" and "Surgical Anatomy of the Arterial Trunks and Fascias" (1837-1839).

The 4th period: academic and military (1840-1856) – was a very active stage of N.I. Pirogov's life, and it should be divided into the following directions: *The first direction: academic and administrative* – working as a professor at the Medical and Surgical Academy in St. Petersburg (1840-1856), founding of the surgical clinic and pathoanatomical museum of the Academy, running the Institute of Practical Anatomy at the Academy (1846), election as a corresponding member of the Russian Academy of Sciences, Biological Branch, and academician of the Medical and Surgical Academy (1847), election as an associate member of the Petersburg Academy of Sciences, election as an honorary member of Moscow, Kharkiv and Kiev universities (1855), retiring from the Academy (1856). *The 2nd direction: military and surgery* – working as a chief doctor at surgical department of II military land hospital, conducting the first surgery under ether anesthesia (1847), working as a military surgeon in the combat army in the Caucasus region (1847), a surgeon and organizer of the military medical staff in the defense of Sevastopol (1854-1855), initiator of involving the Sisters of Charity in the care for the wounded, resigning as a surgeon practitioner (1856). *The 3rd direction: research and practice* – embraced publication of the monograph "On the Transection of the Achilles Tendon as an Operative Orthopedic Therapeutic Agent" (1840), publication of 12 issues of the manual "A Complete Course of Applied

Anatomy of the Human Body, with Figures (Descriptive, Physiological and Surgical Anatomy)" (1843-1848), publication the book "Anatomical Images of the Human Body, Intended Primarily for Forensic Doctors. With an Atlas" (1846), publication of "Illustrated Topographic Anatomy of Cuts Drawn in Three Directions Through a Frozen Human Body" (1852-1859), and publication of the article "Issues of Life" in *Maritime Collection* (1856). *The 5th direction: teaching (September 1856-1861)* – working as a trustee of Odesa (1856) and Kyiv (1858) educational districts, publication of "Collections of Literary Articles by N.I. Pirogov" (1858), publication of the article "What do We Want?" and "On the Subjects of Judgments and Debates at the Pedagogical Councils of Gymnasiums" (1859), "Thoughts and Comments on the Draft Charter of Schools at the Department of Ministry of Public Enlightenment" and "School and Life" (1860), retiring from the office of the trustee due to state of health (1861), moving to Vyshnia village near Vinnytsia (1861). *The 6th direction: scientific and organizational (1862-1866)* – working in Germany as a supervisor of professorial institute, writing scientific and pedagogical articles "The University Issue" and "On Sunday Schools" (1863), publication of the book "Fundamentals of General Military Field Surgery" (1864 – in German, and 1866 – in Russian). *The 7th period at Vyshnia (1867-1881)* – Pirogov's stay in the manor of Vyshnia village, social and medical activities, inspections of military hospitals during the Franco-Prussian War as a representative of the Society of care for the wounded and sick soldiers (1870), publication of "The Report on the Visit to Military Health Facilities in Germany, Lorraine and Alsace in 1870", staying six months on the Russo-Turkish war (1877), writing of "Military and Medical Field and Private Assistance in the War Theater of Bulgaria and in the Rear of the Active Army in 1877-1878" (1879), working on memoirs "The Diary of an Old Doctor" (1879-1881). The proposed periodization of the scientific and pedagogical heritage of doctor N.I. Pirogov on the basis of systematic analysis of different sources reveals new facets of medical and pedagogical activity of the scientist.

CONCLUSIONS

Thus, we suggest to identify the following chronological boundaries in the scientific and educational heritage of the scientist, doctor, educator Pirogov: the lower limit (1824) is the admission to study at the medical faculty of Moscow University; the upper limit (1881) is the scientist's death in his estate in Vyshnia village. The scientific and pedagogical heritage of doctor N.I. Pirogov retains applicability in the modern medicine and education. His medical researches in the field of anatomy, surgery, therapy and phthisiology are still relevant. Pedagogical views of the scientist on the development of secondary and higher education are constantly cited by young researchers. His talent and scientific achievements can only be exceeded by his humanity, unselfishness and humanity.

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REVIEW ARTICLE

NOWY STATUS FIZJOTERAPEUTY W POLSKIM SYSTEMIE OCHRONY ZDROWIA

NEW PHYSIOTHERAPIST STATUS IN THE POLISH HEALTH CARE SYSTEM

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STRESZCZENIE

W Polsce wykonuje zawód obecnie ponad 66 tysięcy fizjoterapeutów. Przez wiele lat zawód fizjoterapeuty nie był uregulowany ustawowo. Od 31 maja 2016 roku obowiązuje ustawa o zawodzie fizjoterapeuty. Ponadto, w 2019 fizjoterapeuci uzyskali nowe uprawnienia w zakresie rehabilitacji leczniczej finansowanej przez Narodowy Fundusz Zdrowia. Obecnie mogą samodzielnie dobierać/zlecać zabiegi oraz zlecać wyroby medyczne na warunkach prawem określonych.

Celem artykułu jest przedstawienie i analiza zmian w prawie mających wpływ na nowy status fizjoterapeuty w systemie ochrony zdrowia w Polsce. Ponadto zwrócenie uwagi na zmianę kompetencji lekarzy w zakresie rehabilitacji leczniczej i zasady ich współpracy z fizjoterapeutami.

Wnioski: Z analizy wynika, że zmiany prawa w ostatnich latach istotnie wpłynęły na wzrost roli fizjoterapeutów w systemie ochrony zdrowia, a także mają one znaczenie dla wykonywania zawodu lekarza.

SŁOWA KLUCZOWE: fizjoterapeuta, ustawa, uprawnienia, skierowanie, lekarz, prawo

ABSTRACT

In Poland, over 66,000 physiotherapists are practicing. For many years, the profession of physiotherapist was not regulated by law. The Act on the profession of physiotherapist has been in force since May 31, 2016. In 2019, physiotherapists obtained new rights in the field of therapeutic rehabilitation financed by the National Health Fund. Currently, they can already choose / order rehabilitation and order medical devices under the conditions of the law.

The aim of the article is to present and analyze changes in law affecting the new status of a physiotherapist in the healthcare system in Poland. In addition, drawing attention to changes in the competence of doctors in the field of therapeutic rehabilitation and the principles of their cooperation with physiotherapists.

Conclusions: The analysis shows that changes in law in recent years have significantly influenced the increase in the role of physiotherapists in the health care system, and they are also important for practicing the medical profession.

KEY WORDS: physiotherapist, statute, authorization, referral, doctor, law

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WSTĘP

Fizjoterapeuci to trzecia (po pielęgniarkach i lekarzach) pod względem liczebności grupa zawodowa personelu medycznego w Polsce. W Krajowym Rejestrze Fizjoterapeutów na dzień 15 stycznia 2020 roku zarejestrowanych było ponad 66 tysięcy osób [1]. Fizjoterapeuci od lat uważani byli za zawód medyczny i funkcjonowali w polskim systemie ochrony zdrowia, mimo braku szczegółowych regulacji prawnych dla tego zawodu. Przez wiele lat nie posiadali własnej pragmatyki ustawowej (ustawy), a ich działalność poza sferą publicznego ubezpieczenia zdrowotnego (gdzie określone były i są wymogi dla świadczeń gwarantowanych w zakresie rehabilitacji leczniczej) nie podlegała zasadniczo żadnym specyficznym dla zawodu regulacjom prawnym. Z uwagi na swój przedmiot oraz bezpieczeństwo pacjentów rehabilitacja lecznicza, a przede

wszystkim zasady wykonywania zawodu fizjoterapeuty, wymagały od dawna kompleksowego uregulowania ustawowego. Także samo środowisko fizjoterapeutów postulowało od lat uregulowanie swojego zawodu. Ostatecznie 25 września 2015 roku uchwalona została ustawa o zawodzie fizjoterapeuty (t. jedn. Dz. U. 2019; poz.952) zwana w skrócie u.z.f. Powyższa ustawa weszła w życie 31 maja 2016 roku. Ustawa z dnia 25 września 2015 roku o zawodzie fizjoterapeuty określa zasady:

1. wykonywania zawodu fizjoterapeuty,
2. uzyskiwania prawa wykonywania zawodu fizjoterapeuty,
3. kształcenia zawodowego fizjoterapeutów,
4. kształcenia podyplomowego fizjoterapeutów,
5. odpowiedzialności zawodowej fizjoterapeutów.

Ustawa przedmiotowa określa również organizację i zadania samorządu zawodowego fizjoterapeutów oraz prawa i obowiązki

jego członków. Na podstawie powyższej ustawy utworzono po raz pierwszy w historii Polski samorząd zawodowy fizjoterapeutów. Samorząd zawodowy fizjoterapeutów, podobnie jak samorząd zawodowy lekarzy reprezentuje osoby wykonujące zawód fizjoterapeuty oraz sprawuje pieczę nad należyтым wykonywaniem tego zawodu w granicach interesu publicznego i dla jego ochrony [2]. Przynależność fizjoterapeutów do samorządu jest obowiązkowa. Jednostką organizacyjną samorządu, posiadającą osobowość prawną jest Krajowa Izba Fizjoterapeutów z siedzibą w Warszawie (KIF). KIF tworzą fizjoterapeuci wpisani do Krajowego Rejestru Fizjoterapeutów.

Fizjoterapeuci to obecnie zawód regulowany ustawowo i posiadający własny samorząd zawodowy. Od czasu wejścia w życie ustawy o zawodzie fizjoterapeuty wzmocnieniu uległa ich pozycja zawodowa w systemie ochrony zdrowia. Dodatkowo w roku 2019 znacznemu poszerzeniu uległy ich kompetencje w sferze świadczeń gwarantowanych w systemie ubezpieczenia zdrowotnego. Na zasadach określonych prawem fizjoterapeuci mogą bowiem samodzielnie dobierać pacjentom zabiegi fizjoterapii, a także zlecać wyroby medyczne. Nowe regulacje dotyczące rehabilitacji leczniczej mają wpływ na wykonywanie zawodu lekarza, a w szczególności zmieniają zasady wystawiania skierowań na rehabilitację ambulatoryjną i domową.

W przepisach prawa ochrony zdrowia nie ma definicji rehabilitacji leczniczej. Termin „rehabilitacja” oznacza dosłownie przywracanie sprawności. Definicja normatywna rehabilitacji w polskim systemie prawnym znajduje się w Ustawie z dnia 27 sierpnia 1997 r. o rehabilitacji zawodowej i społecznej oraz zatrudnianiu osób niepełnosprawnych (t. jedn. Dz.U. 2018; poz. 511). Zgodnie z art. 7 powyższej ustawy rehabilitacja to zespół działań, zwłaszcza organizacyjnych, leczniczych, psychologicznych, technicznych, szkoleniowych, edukacyjnych i społecznych, zmierzających do osiągnięcia przy aktywnym uczestnictwie tych osób możliwie najwyższego poziomu ich funkcjonowania, jakości życia i integracji społecznej. Powszechnie przyjmuje się podział rehabilitacji na:

1. rehabilitację medyczną (leczniczą),
2. rehabilitację zawodową,
3. rehabilitację społeczną.

W piśmiennictwie medycznym terminem odnoszącym się do rehabilitacji jest rehabilitacja medyczna, natomiast w terminologii normatywnej związanej z systemem publicznego ubezpieczenia zdrowotnego funkcjonuje pojęcie rehabilitacja lecznicza [3]. Należy przyjąć, że rehabilitacja medyczna (zwana też leczniczą) jest postępowaniem, które umożliwia przyspieszenie procesu naturalnej regeneracji i plastyczności (dla układu nerwowego) oraz zmniejszenie następstw ustrojowych i psychicznych spowodowanych przez chorobę lub uraz [4]. Powszechnie przyjmuje się, że rehabilitacja medyczna (lecznicza) obejmuje trzy podstawowe działy (dziedziny) t.j.: kinezyterapię, fizykoterapię oraz masaż.

CEL PRACY

Celem głównym niniejszego artykułu jest przedstawienie nowego statusu fizjoterapeuty jako zawodu ustawowo

regulowanego w systemie ochrony zdrowia w Polsce, ze szczególnym uwzględnieniem poszerzenia zakresu kompetencji w sferze świadczeń gwarantowanych w systemie ubezpieczenia zdrowotnego. Celem szczegółowym jest zwrócenie uwagi na zmianę kompetencji lekarzy w zakresie rehabilitacji leczniczej, a także przedstawienie i analiza zasad ich współpracy w fizjoterapeutami w systemie publicznego ubezpieczenia zdrowotnego.

MATERIAŁ I METODY

W pracy dokonano przede wszystkim analizy aktualnie obowiązujących norm prawnych dotyczących wykonywania zawodu fizjoterapeuty oraz rehabilitacji leczniczej, jako świadczenia gwarantowanego w systemie ubezpieczenia zdrowotnego. W szczególności poddano analizie ustawę z dnia 25 września 2015 roku o zawodzie fizjoterapeuty (t. jedn. Dz. U. 2019; poz.952), a także rozporządzenie Ministra Zdrowia z dnia 6 listopada 2013 r. w sprawie świadczeń gwarantowanych z zakresu rehabilitacji leczniczej (t. jedn. Dz. U. 2018; poz. 465 z późn. zm.). W pracy zastosowano metodę analityczno-syntetyczną a także wykorzystano dane statystyczne z rejestru fizjoterapeutów.

PRZEGLĄD

PRAWO WYKONYWANIA ZAWODU FIZJOTERAPEUTY

Jednym z instrumentów ustawowych regulujących rynek działalności leczniczej oraz będącym standardem dla większości podstawowych zawodów medycznych jest konieczność posiadania prawa wykonywania zawodu medycznego [5]. Powyższe prawo przyznawane jest przez uprawnione organy samorządu zawodowego osobom posiadającym odpowiednie wykształcenie zawodowe oraz spełniającym inne przesłanki określone ustawowo.

Po wejściu w życie przepisów u.z.f. samo ukończenie adekwatnych studiów nie uprawnia do wykonywania zawodu fizjoterapeuty, a w szczególności do udzielania świadczeń zdrowotnych. Elementem niezbędnym do wykonywania zawodu fizjoterapeuty jest uzyskanie tzw. prawa wykonywania zawodu. Zgodnie z art. 2 u.z.f. zawód fizjoterapeuty jest samodzielnym zawodem medycznym. Prawo posługiwania się tytułem zawodowym „fizjoterapeuta” przysługuje osobie posiadającej prawo wykonywania zawodu. Nie można więc używać powyższego tytułu bez posiadania takiego prawa. Zgodnie z art. 13 u.z.f. prawo wykonywania zawodu fizjoterapeuty przysługuje osobie, która spełnia łącznie następujące warunki:

1. ma pełną zdolność do czynności prawnych;
2. jej stan zdrowia pozwala na wykonywanie zawodu fizjoterapeuty, co potwierdza się orzeczeniem lekarskim albo innym dokumentem potwierdzającym brak przeciwwskazań do wykonywania zawodu fizjoterapeuty wymaganych w państwie członkowskim Unii Europejskiej innym niż Rzeczpospolita Polska lub Konfederacji Szwajcarskiej lub w państwie członkowskim Europej-

skiego Porozumienia o Wolnym Handlu (EFTA) – stronie umowy o Europejskim Obszarze Gospodarczym wydanym przez właściwe podmioty w tym państwie;

3. wykazuje znajomość języka polskiego w stopniu wystarczającym do wykonywania zawodu fizjoterapeuty;
4. swoim dotychczasowym zachowaniem daje rękojmię prawidłowego wykonywania zawodu fizjoterapeuty, w szczególności nie była skazana prawomocnym wyrokiem za umyślne przestępstwo przeciwko życiu i zdrowiu, przeciwko wolności seksualnej i obyczajności oraz za czyny określone w art. 207 i art. 211 ustawy z dnia 6 czerwca 1997 r. – Kodeks karny (Dz. U. z 2018 r. poz. 1600 i 2077);
5. posiada odpowiedni dyplom, świadectwo lub inny dokument potwierdzający kwalifikacje do wykonywania zawodu fizjoterapeuty.

Prawo wykonywania zawodu stwierdza, na wniosek osoby zainteresowanej, Krajowa Rada Fizjoterapeutów. Na podstawie uchwał o stwierdzeniu lub przyznaniu prawa wykonywania zawodu Krajowa Rada Fizjoterapeutów dokonuje wpisu do Krajowego Rejestru Fizjoterapeutów i wydaje dokument „Prawo wykonywania zawodu fizjoterapeuty”. Zgodnie z art. 26 u.z.f. prawo wykonywania zawodu fizjoterapeuty wygasa w przypadku: śmierci, zrzeczenia się prawa wykonywania zawodu, prawomocnego orzeczenia przez sąd dyscyplinarny zakazu wykonywania zawodu, utraty pełnej zdolności do czynności prawnych.

Krajowa Izba Fizjoterapeutów (tak jak Izby Lekarskie) jest uprawniona w określonych ustawowo sytuacjach do ingerencji w prawo wykonywania zawodu fizjoterapeuty (zawieszenie, ograniczenie, pozbawienie PWZF) [6]. Ingerować w PWZF może także prokurator i sąd w postępowaniu karnym (środek zapobiegawczy, środek karny).

Fizjoterapeuci mieli obowiązek uzyskania prawa wykonywania zawodu najpóźniej do dnia 31 maja 2018 roku. Brak PWZF wywołuje wiele skutków prawnych, w szczególności zakaz wykonywania pracy i świadczenia usług w charakterze fizjoterapeuty, a także odpowiedzialność karną. W praktyce osoba nieposiadająca PWZF, mimo odpowiedniego wykształcenia i doświadczenia zawodowego, nie może legalnie wykonywać żadnej czynności zawodowej na rzecz pacjenta/klienta w zakresie fizjoterapii/rehabilitacji leczniczej. Art. 28 u.z.f. wyraźnie stanowi, że fizjoterapeuta przed rozpoczęciem wykonywania zadań zawodowych na podstawie umowy o pracę lub umowy cywilnoprawnej jest zobowiązany przedłożyć podmiotowi, na rzecz którego wykonuje te zadania, dokument potwierdzający prawo wykonywania zawodu. W praktyce każdy pracodawca (szpital, przychodnia itp.), a także podmiot, z którym łączy fizjoterapeutę umowa cywilna (np. zlecenia lub o świadczenie usług z zakresu fizjoterapii), ma obowiązek żądać przedstawienia prawa wykonywania zawodu przez każdego fizjoterapeutę. Poza skutkami w zakresie możliwości podjęcia pracy brak prawa wykonywania zawodu skutkuje odpowiedzialnością karną. Odpowiedzialność karna dotyczy zarówno samego fizjoterapeuty, a także osoby go zatrudniającej. Sankcjonowane jest nieuprawnione posługiwanie się tytułem zawodowym fizjoterapeuty oraz

udzielanie świadczeń bez uprawnień, a także dopuszczenie osoby nieuprawnionej do udzielania świadczeń z zakresu fizjoterapii [7].

USTAWOWY ZAKRES CZYNNOŚCI ORAZ STANDARDY WYKONYWANIA ZAWODU FIZJOTERAPEUTY

Ustawa o zawodzie fizjoterapeuty nie zawiera typowej definicji legalnej pojęcia „fizjoterapeuta”. Wskazuje natomiast bezpośrednio na elementy (czynności) wchodzące w zakres wykonywania tego zawodu. Ustawa określa tym samym przedmiot-zakres wykonywania zawodu fizjoterapeuty. Generalnie wykonywanie zawodu fizjoterapeuty w świetle nowej ustawy polega na udzielaniu świadczeń zdrowotnych przez osobę mającą odpowiednie kwalifikacje i prawo wykonywania zawodu. Pojęcie świadczeń zdrowotnych zdefiniowane zostało w ustawie z dnia 15 kwietnia 2011 r. o działalności leczniczej (t. jedn. Dz.U. 2018; poz.2190). Zgodnie z art.3. powyższej ustawy działalność lecznicza polega na udzielaniu świadczeń zdrowotnych, a świadczenia zdrowotne są to działania służące zachowaniu, ratowaniu, przywracaniu lub poprawie zdrowia oraz inne działania medyczne wynikające z procesu leczenia lub przepisów odrębnych regulujących zasady ich wykonywania. Ogólny wykaz świadczeń zdrowotnych, do których wykonywania uprawniony jest fizjoterapeuta, zawiera art. 4 ust. 2 u.z.f. Zgodnie z tym artykułem wykonywanie zawodu fizjoterapeuty polega na udzielaniu świadczeń zdrowotnych, w szczególności na:

1. diagnostyce funkcjonalnej pacjenta;
2. kwalifikowaniu, planowaniu i prowadzeniu fizykoterapii;
3. kwalifikowaniu, planowaniu i prowadzeniu kinezyterapii;
4. kwalifikowaniu, planowaniu i prowadzeniu masażu;
5. zlecaniu wyrobów medycznych, zgodnie z przepisami wydanymi na podstawie ustawy z dnia 12 maja 2011 r. o refundacji leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych (t. jedn. Dz. U. 2019; poz.784);
6. dobieraniu do potrzeb pacjenta wyrobów medycznych;
7. nauczaniu pacjentów posługiwania się wyrobami medycznymi;
8. prowadzeniu działalności fizjoprofilaktycznej, polegającej na popularyzowaniu zachowań prozdrowotnych oraz kształtowaniu i podtrzymywaniu sprawności i wydolności osób w różnym wieku w celu zapobiegania niepełnosprawności;
9. wydawaniu opinii i orzeczeń odnośnie do stanu funkcjonalnego osób poddawanych fizjoterapii oraz przebiegu procesu fizjoterapii;
10. nauczaniu pacjentów mechanizmów kompensacyjnych i adaptacji do zmienionego potencjału funkcji ciała i aktywności.

Z uwagi na przyjęty przez ustawodawcę zwrot w szczególności należy stwierdzić, iż powyższy wykaz czynności-swiadczeń nie ma charakteru zamkniętego. Wyżej wymienionych świadczeń zdrowotnych może udzielać samodzielnie fizjoterapeuta posiadający odpowiedni tytuł

(magistra, licencjata lub technika fizjoterapii) oraz odpowiednio doświadczenie zawodowe (co najmniej 3-letnie w przypadku magistrów oraz 6-letnie w przypadku licencjatów i techników fizjoterapii). W świetle ustawy wszystkich wyżej wskazanych (tj. punkty od 1 do 10) świadczeń zdrowotnych może udzielać samodzielnie fizjoterapeuta posiadający tytuł:

1. magistra uzyskany w sposób wskazany w ustawie (czyli taka osoba, która rozpoczęła po dniu 1 października 2017 r. jednolite 5-letnie studia wyższe w zakresie fizjoterapii, obejmujące co najmniej 300 punktów ECTS oraz odbyła 6-miesięczną praktykę zawodową i uzyskała tytuł zawodowy magistra oraz złożyła Państwowy Egzamin Fizjoterapeutyczny z wynikiem pozytywnym);
2. magistra uzyskany w sposób wskazany w ustawie (art. 13 ust. 3 pkt 2–7) oraz co najmniej 3-letnie doświadczenie w zawodzie fizjoterapeuty.

Natomiast części wyżej wskazanych świadczeń zdrowotnych, tj. polegających na:

1. diagnostyce funkcjonalnej pacjenta,
2. planowaniu i prowadzeniu fizykoterapii,
3. planowaniu i prowadzeniu kinezyterapii,
4. kwalifikowaniu, planowaniu i prowadzeniu masażu,
5. dobieraniu do potrzeb pacjenta wyrobów medycznych,
6. nauczaniu pacjentów posługiwania się wyrobami medycznymi,
7. prowadzeniu działalności fizjoprofilaktycznej,
8. nauczaniu pacjentów mechanizmów kompensacyjnych i adaptacji do zmienionego potencjału funkcji ciała i aktywności;

może udzielać samodzielnie fizjoterapeuta posiadający tytuł:

- a. licencjata uzyskany w sposób wskazany w ustawie oraz co najmniej 6-letnie doświadczenie w zawodzie fizjoterapeuty,
- b. zawodowy technika fizjoterapii uzyskany w sposób wskazany w ustawie oraz co najmniej 6-letnie doświadczenie w zawodzie fizjoterapeuty.

Podkreślić należy, że osoby posiadające tytuł licencjata lub technika fizjoterapii mają zawężone uprawnienia w zakresie wykonywania świadczeń zdrowotnych – bowiem nie mogą one kwalifikować do fizykoterapii i kinezyterapii, zlecać wyrobów medycznych, a także wydawać opinii i orzeczeń odnośnie stanu funkcjonalnego osób poddawanych fizjoterapii oraz przebiegu procesu fizjoterapii.

Szczegółowy wykaz świadczeń udzielanych przez fizjoterapeutów określa rozporządzenie Ministra Zdrowia z dnia 27 czerwca 2018 r. w sprawie szczegółowego wykazu czynności zawodowych fizjoterapeuty (Dz. U. z 2019; poz. 1319). Minister Zdrowia określił w załączniku do powyższego rozporządzenia szczegółowy wykaz czynności zawodowych fizjoterapeuty w ramach poszczególnych zadań zawodowych i odpowiadający im poziom wykształcenia, niezbędny do ich wykonywania. W przedmiotowym rozporządzeniu określono trzy poziomy wykształcenia fizjoterapeuty, tj.: podstawowy, rozszerzony, specjalistyczny [8].

Ustawa o zawodzie fizjoterapeuty poza wskazaniem czynności zawodowych określa także podstawowe standardy prawne wykonywania zawodu fizjoterapeuty. Przede

wszystkim w świetle art. 4 ust. 1 u.z.f. fizjoterapeuta powinien wykonywać swój zawód z należytą starannością, zgodnie z zasadami etyki zawodowej, poszanowaniem praw pacjenta, dbałością o jego bezpieczeństwo i wykorzystując wskazania aktualnej wiedzy medycznej. Podobna formuła ustawowa dotyczy lekarzy.

Przy wykonywaniu zawodu fizjoterapeuta współpracuje z osobami wykonującymi inne zawody medyczne, a w szczególności z lekarzami. Co bardzo istotne w przypadku uzasadnionych wątpliwości odnoszących się do zleceń lekarza w zakresie fizjoterapii, fizjoterapeuta ma prawo domagać się od lekarza, który wydał zlecenie, by uzasadnił potrzebę jego wykonania, a także prawo odmowy wykonania określonego świadczenia. Odmowę wykonania świadczenia fizjoterapeuta uzasadnia w dokumentacji medycznej i informuje o niej lekarza zlecającego (art. 6 ust. 2 u.z.f.). Ponadto fizjoterapeuta ma prawo wglądu do dokumentacji medycznej pacjenta oraz do uzyskania od podmiotu udzielającego świadczeń zdrowotnych pełnej informacji o stanie zdrowia pacjenta, rozpoznaniu, proponowanych metodach diagnostycznych, leczniczych, rehabilitacyjnych, zapobiegawczych i dających się przewidzieć następstwach podejmowanych działań w zakresie niezbędnym do udzielanych przez siebie świadczeń zdrowotnych. W praktyce lekarz powinien udzielić fizjoterapeucie informacji o stanie zdrowia pacjenta i umożliwić wgląd do jego dokumentacji medycznej.

Istotną częścią wykonywania zawodu fizjoterapeuty, a teraz również standardem ustawowym wynikającym z własnej pragmatyki zawodowej, jest obowiązek przestrzegania praw pacjenta. Realizacja praw pacjenta należy do podstawowych obowiązków prawnych każdego podmiotu wykonującego działalność leczniczą. Podstawowym aktem prawnym regulującym prawa polskiego pacjenta jest ustawa z dnia 6 listopada 2008 r. o prawach pacjenta i Rzeczniku Praw Pacjenta (t. jedn. Dz.U. 2019; poz. 1127 z późn. zm.). Zgodnie z art. 9 u.z.f. fizjoterapeuta jest zobowiązany:

1. informować pacjenta o jego prawach określonych w przepisach ustawy z dnia 6 listopada 2008 r. o prawach pacjenta i Rzeczniku Praw Pacjenta;
2. udzielać informacji pacjentowi lub jego przedstawicielowi ustawowemu albo osobie bliskiej lub opiekunowi faktycznemu w zakresie niezbędnym do udzielanych przez siebie świadczeń zdrowotnych;
3. zachowywać w tajemnicy informacje związane z pacjentem, uzyskane w związku z wykonywaniem zawodu zgodnie z przepisami ustawy z dnia 6 listopada 2008 r. o prawach pacjenta i Rzeczniku Praw Pacjenta;
4. prowadzić i udostępniać dokumentację medyczną na zasadach określonych w ustawie z dnia 6 listopada 2008 r. o prawach pacjenta i Rzeczniku Praw Pacjenta.

W rezultacie do podstawowych ustawowych obowiązków fizjoterapeuty stanowiących korelat praw pacjenta należą: obowiązek informacyjny, obowiązek przestrzegania tajemnicy zawodowej oraz obowiązki w zakresie dokumentacji medycznej.

Art. 8 u.z.f. ustanawia specyficzny instrument kontroli pracy fizjoterapeutów, z którego w praktyce mogą korzystać tak-

że lekarze. Bowiem zgodnie z powyższym artykułem, jeżeli osoba wykonująca zawód medyczny stwierdziła naruszenie zasad wykonywania zawodu fizjoterapeuty, jest obowiązana niezwłocznie przekazać swoje uwagi osobie naruszającej zasady wykonywania zawodu. W przypadku gdy działanie wyżej wskazane okaże się nieskuteczne, a dalsze naruszanie zasad wykonywania zawodu fizjoterapeuty może prowadzić do zagrożenia życia lub zdrowia pacjenta, osoba wykonująca zawód medyczny niezwłocznie zawiadamia o tym bezpośredniego przełożonego oraz Rzecznika Dyscyplinarnego.

SKIEROWANIE NA REHABILITACJĘ LECZNICZĄ

Świadczeniobiorca ma prawo do rehabilitacji leczniczej jako świadczenia gwarantowanego w systemie publicznego ubezpieczenia zdrowotnego. Ogólnie prawo świadczeniobiorcy do świadczeń zdrowotnych w zakresie rehabilitacji leczniczej reguluje art. 15 ust. 2 pkt 5 ustawy z dnia 27 sierpnia 2004 r. o świadczeniach opieki zdrowotnej finansowanych ze środków publicznych (t.jedn. Dz.U. 2019; poz.1373) zwanej w skrócie u.ś.o.z., a szczegółowo rozporządzenie Ministra Zdrowia z dnia 6 listopada 2013 r. w sprawie świadczeń gwarantowanych z zakresu rehabilitacji leczniczej (t. jedn. Dz. U. 2018; poz. 465 z późn. zm.). Świadczenia gwarantowane z rehabilitacji leczniczej są realizowane w warunkach:

1. ambulatoryjnych,
2. domowych,
3. ośrodka lub oddziału dziennego, które obejmują rehabilitację:
 - a. ogólnoustrojową, w tym dla określonych grup pacjentów,
 - b. dzieci z zaburzeniami wieku rozwojowego,
 - c. osób z dysfunkcją narządu słuchu i mowy,
 - d. osób z dysfunkcją narządu wzroku,
 - e. kardiologiczną lub kardiologiczną telerehabilitację hybrydową,
 - f. pulmonologiczną z wykorzystaniem metod subteranneoterapii,
4. stacjonarnych, które obejmują rehabilitację:
 - a. ogólnoustrojową,
 - b. neurologiczną,
 - c. pulmonologiczną,
 - d. kardiologiczną lub kardiologiczną telerehabilitację hybrydową [9].

W rezultacie świadczeniobiorcy przysługują świadczenia rehabilitacji leczniczej udzielane w warunkach ambulatoryjnych, domowych, ośrodka dziennego oraz stacjonarnych. Świadczeniobiorca ma prawo do rehabilitacji leczniczej u świadczeniodawcy, który zawarł umowę o udzielanie świadczeń opieki zdrowotnej, na podstawie skierowania lekarza ubezpieczenia zdrowotnego.

Od 1 stycznia 2019 uległy istotnej zmianie zasady dotyczące wystawiania skierowań i dobierania zabiegów realizowanych w warunkach ambulatoryjnych i domowych. Świadczenia gwarantowane w zakresie rehabilitacji realizowane w warunkach ambulatoryjnych obejmują:

1. lekarską ambulatoryjną opiekę rehabilitacyjną (jest to porada lekarska rehabilitacyjna),

2. fizjoterapię ambulatoryjną: wizyta fizjoterapeutyczna i zabieg fizjoterapeutyczny (zabiegi z zakresu kinezyterapii, fizykoterapii oraz masażu).

Fizjoterapia ambulatoryjna odbywa się na podstawie skierowania wydanego przez lekarza ubezpieczenia zdrowotnego w cyklu terapeutycznym do 10 dni zabiegowych, jednemu świadczeniobiorcy przysługuje nie więcej niż 5 zabiegów dziennie. Skierowanie na fizjoterapię w warunkach ambulatoryjnych wystawić może każdy lekarz ubezpieczenia zdrowotnego (zarówno POZ, jak i specjalista np. neurolog, ortopeda).

Świadczenia gwarantowane realizowane w warunkach domowych obejmują:

- a. poradę lekarską rehabilitacyjną,
- b. fizjoterapię domową realizowaną przez: wizytę fizjoterapeutyczną i zabieg fizjoterapeutyczny.

Fizjoterapia domowa to świadczenia udzielane świadczeniobiorcom, którzy ze względu na brak możliwości samodzielnego poruszania się nie mogą dotrzeć do świadczeniobiorcy o dawców udzielających świadczeń gwarantowanych o charakterze ambulatoryjnym, a wymagają rehabilitacji leczniczej. Przeznaczona jest dla pacjentów z zaburzeniami funkcji motorycznych spowodowanych określonymi chorobami, a także w określonym przedziale czasowym. Zabiegi fizjoterapeutyczne w warunkach domowych są udzielane świadczeniobiorcom z zaburzeniami funkcji motorycznych spowodowanymi:

1. ogniskowymi uszkodzeniami mózgu (stanami po zatorach mózgowych, udarach krwotocznych mózgu, urazach) – w okresie do 12 miesięcy od dnia powstania ogniskowego uszkodzenia mózgu,
2. ciężkimi uszkodzeniami centralnego i obwodowego układu nerwowego odpowiadającymi 5. stopniowi skali oceny stopnia niepełnosprawności, zwanej „skalą opartą na skali Rankina” (warunku otrzymania 5. stopnia skali opartej na skali Rankina nie stosuje się do dzieci do ukończenia 18. roku życia),
3. uszkodzeniem rdzenia kręgowego – w okresie do 12 miesięcy od dnia powstania uszkodzenia rdzenia kręgowego,
4. chorobami przewlekłe postępującymi, w szczególności: miopatiami, chorobą Parkinsona, zapaleniem wielomięśniowym, rdzeniowym zanikiem mięśni, guzami mózgu, procesami demielinizacyjnymi, kolagenozami, przewlekłymi zespołami pozapiramidowymi, reumatoidalnym zapaleniem stawów,
5. chorobami zwyrodnieniowymi stawów biodrowych lub kolanowych oraz po zabiegach endoprotezoplastyki stawu – w okresie do 6 miesięcy od dnia wykonania operacji,
6. złamaniami, obrażeniami i amputacjami kończyn dolnych – w okresie do 6 miesięcy od dnia złamania, obrażenia lub amputacji,
7. osobom w stanie wegetatywnym lub apalicznym.

Czas trwania rehabilitacji realizowanej w warunkach domowych dla jednego świadczeniobiorcy wynosi do 80 dni zabiegowych w roku kalendarzowym oraz nie więcej niż 5 zabiegów dziennie. Skierowanie na rehabilitację do-

nową jest wystawiane przez lekarza podstawowej opieki zdrowotnej lub innego lekarza ubezpieczenia zdrowotnego:

- a. specjalistę w dziedzinie: rehabilitacji w chorobach narządu ruchu lub rehabilitacji ogólnej, lub rehabilitacji, lub rehabilitacji medycznej, medycyny fizykalnej i balneoklimatologii, lub fizjoterapii i balneoklimatologii, lub balneoklimatologii i medycyny fizykalnej, lub balneologii, lub balneologii i medycyny fizykalnej, reumatologii, neurologii, neurochirurgii lub neurochirurgii i neurotraumatologii, chirurgii, lub chirurgii ogólnej, chirurgii ortopedycznej, lub chirurgii urazowo-ortopedycznej, lub ortopedii i traumatologii, lub ortopedii i traumatologii narządu ruchu, lub
- b. lekarza ze specjalizacją I stopnia w dziedzinie: rehabilitacji w chorobach narządu ruchu lub rehabilitacji ogólnej, lub rehabilitacji, lub rehabilitacji medycznej, medycyny fizykalnej i balneoklimatologii, neurologii, neurochirurgii, chirurgii ogólnej, ortopedii i traumatologii, lub
- c. lekarza w trakcie specjalizacji w dziedzinie: rehabilitacji medycznej, balneologii i medycyny fizykalnej, reumatologii, neurologii, neurochirurgii, chirurgii ogólnej, ortopedii i traumatologii narządu ruchu.

Po zmianie przepisów od 1 stycznia 2019 skierowanie lekarskie na rehabilitację w warunkach ambulatoryjnych, tak jak i na rehabilitację w warunkach domowych, powinno zawierać:

- a. pieczęć nagłówkową z numerem umowy zawartej z dyrektorem właściwego oddziału wojewódzkiego Narodowego Funduszu Zdrowia,
- b. imię, nazwisko, adres zamieszkania świadczeniobiorcy oraz numer PESEL, a w przypadku braku numeru PESEL – numer dokumentu potwierdzającego tożsamość,
- c. rozpoznanie w języku polskim,
- d. kod jednostki chorobowej według Klasyfikacji ICD-10,
- e. choroby przebyte i współistniejące oraz inne czynniki, w tym wszczepione urządzenia wspomagające pracę układu krążeniowo-oddechowego lub metal w ciele pacjenta albo przyjmowanie niektórych leków, mogące mieć istotny wpływ na proces rehabilitacji lub na postępowanie fizjoterapeutyczne,
- f. pieczęć i podpis lekarza wystawiającego to skierowanie oraz datę jego wystawienia.

W przypadku skierowania wystawionego przez lekarza ubezpieczenia zdrowotnego będącego lekarzem specjalistą w dziedzinie: rehabilitacji ogólnej lub rehabilitacji medycznej czy rehabilitacji w chorobach narządu ruchu skierowanie to może zawierać zlecone zabiegi fizjoterapeutyczne oraz liczbę poszczególnych zabiegów w cyklu zabiegów. Jednakże w przypadku uzasadnionych wątpliwości odnoszących się do zleconych przez powyższego specjalistę zabiegów fizjoterapeutycznych fizjoterapeuta może dokonać zmiany w tym zakresie po konsultacji z lekarzem i odnotowaniu tego faktu w dokumentacji medycznej!

W rezultacie zmian rozporządzenia o świadczenia gwarantowanych z zakresu rehabilitacji leczniczej lekarz kierujący w praktyce **nie wpisuje już w skierowaniu rodzaju (nazw) zleconych zabiegów i ich liczby**. Bowiem o rodzaju i liczbie zabiegów (w granicy przysługującej dla

danego świadczenia gwarantowanego) będzie decydował uprawniony fizjoterapeuta z zakładu rehabilitacji leczniczej, do którego trafi pacjent [10].

Zlecenie zabiegów przez fizjoterapeutę powinno być związane z tzw. wizytą fizjoterapeutyczną. Wizyta fizjoterapeutyczna stanowi samodzielny element świadczeń gwarantowanych fizjoterapii w warunkach ambulatoryjnych i domowych. Obejmować ona może m.in. zaplanowanie postępowania fizjoterapeutycznego oraz ocenę i opis stanu funkcjonalnego świadczeniobiorcy. Fizjoterapeuta realizuje wizytę fizjoterapeutyczną generalnie na początku i na końcu danego cyklu zabiegów. Do realizacji wizyty fizjoterapeutycznej (warunki ambulatoryjne i domowe) nie jest uprawniony każdy fizjoterapeuta. W świetle załącznika nr 1 do rozporządzenia Ministra Zdrowia z dnia 6 listopada 2013 r. w sprawie świadczeń gwarantowanych z zakresu rehabilitacji leczniczej może ją zrealizować tylko fizjoterapeuta posiadający tytuł specjalisty w dziedzinie fizjoterapii lub tytuł specjalisty rehabilitacji ruchowej II stopnia bądź fizjoterapeuta posiadający tytuł magistra (o którym mowa w art. 4 ust. 4 pkt 1 i 2 ustawy z dnia 25 września 2015 r. o zawodzie fizjoterapeuty) lub odpowiedni dyplom, świadectwo czy inny dokument potwierdzający ukończenie studiów drugiego stopnia oraz posiadający co najmniej 3-letnie doświadczenie w zawodzie fizjoterapeuty.

ZLECENIA NA WYROBY MEDYCZNE

Świadczeniobiorca w ramach świadczeń gwarantowanych ma prawo do zaopatrzenia w wyroby medyczne, na zlecenie osoby uprawnionej, oraz ich naprawy (15 ust. 2 pkt 9 u.s.o.z.). Wyrób medyczny przysługuje raz w danym okresie użytkowania potwierdzonym wskazaniem medycznym. Zgodnie z definicją ustawową zawartą w ustawie z dnia 20 maja 2010 r. o wyrobach medycznych (t. jedn. Dz. U. 2019; poz.175) wyrób medyczny to narzędzie, przyrząd, urządzenie, oprogramowanie, materiał lub inny artykuł, stosowany samodzielnie lub w połączeniu, w tym z oprogramowaniem przeznaczonym przez jego wytwórcę do używania specjalnie w celach diagnostycznych lub terapeutycznych i niezbędnym do jego właściwego stosowania, przeznaczony przez wytwórcę do stosowania u ludzi w celu:

- a) diagnozowania, zapobiegania, monitorowania, leczenia lub łagodzenia przebiegu choroby;
- b) diagnozowania, monitorowania, leczenia, łagodzenia lub kompensowania skutków urazu lub upośledzenia;
- c) badania, zastępowania lub modyfikowania budowy anatomicznej lub procesu fizjologicznego;
- d) regulacji poczęć

- który nie osiąga zasadniczego zamierzonego działania w ciele lub na ciele ludzkim środkami farmakologicznymi, immunologicznymi lub metabolicznymi, lecz którego działanie może być wspomagane takimi środkami.

Wyroby medyczne to bardzo szeroka gama produktów obejmująca zarówno rękawiczki, strzykawki, jak i specjalistyczny sprzęt diagnostyczny (np. tomografy), a także protezy i ortezy. W ramach wyrobów medycznych wydawanych na zlecenie przepisy wyodrębniają m.in. wyrób

medyczny przysługujący comiesięcznie (taki, dla którego okres użytkowania określono „raz na miesiąc”), wyrób medyczny wykonywany na zamówienie oraz wyrób medyczny produkowany seryjnie.

Poza ustawą z dnia 27 sierpnia 2004 r. o świadczeniach opieki zdrowotnej finansowanych ze środków publicznych kwestię zaopatrzenia w wyroby medyczne reguluje ustawa z dnia 12 maja 2011 r. o refundacji leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych (t. jedn. Dz. U. 2019; poz. 784). Rozporządzenie Ministra Zdrowia z dnia 29 maja 2017 r. w sprawie wykazu wyrobów medycznych wydawanych na zlecenie (t. jedn. Dz. U. 2019; poz. 1267) określa:

1. wykaz wyrobów medycznych wydawanych na zlecenie osoby uprawnionej wraz z określeniem limitów ich finansowania ze środków publicznych i wysokości udziału własnego świadczeniobiorcy w tym limicie;
2. kryteria przyznawania wyrobów medycznych wydawanych na zlecenie,
3. okresy użytkowania wyrobów medycznych wydawanych na zlecenie,
4. limity cen napraw wyrobów medycznych wydawanych na zlecenie

- stanowiące załącznik do rozporządzenia.

Wyżej wskazane rozporządzenie zostało w sposób istotny zmienione. Nowelizacja uwzględniająca m.in. fizjoterapeutów jako uprawnionych do wystawiania zleceń na konkretne wyroby medyczne weszła w życie 23 października 2019 roku. Ponadto 1 września 2019 roku weszło w życie rozporządzenie Ministra Zdrowia z dnia 2 sierpnia 2019 r. w sprawie zlecenia na zaopatrzenie w wyroby medyczne oraz zlecenia naprawy wyrobu medycznego (Dz. U. 2019; poz. 1555). Powyższe rozporządzenie zawiera szczegółowe regulacje w zakresie obowiązkowej nowej treści zlecenia na wyroby medyczne.

Osobami uprawnionymi do wystawiania zleceń na wyroby medyczne są przede wszystkim lekarze a także pielęgniarki oraz od niedawna fizjoterapeuci [11]. Samo wykonywanie powyższego zawodu nie uprawnia z reguły do wystawiania zleceń bowiem niezbędne jest posiadanie odpowiedniego poziomu wykształcenia zawodowego (przede wszystkim magisterskiego w przypadku pielęgniarek i fizjoterapeutów) lub specjalizacji. W przypadku lekarzy wymagany jest w większości przypadków określony rodzaj specjalizacji (zależnie od rodzaju wyrobu) ukończonej lub w trakcie np. specjalizacja w dziedzinie: ortopedii i traumatologii lub chirurgii ortopedycznej, lub chirurgii urazowo-ortopedycznej, lub ortopedii i traumatologii narządu ruchu, chirurgii ogólnej, chirurgii naczyniowej, chirurgii dziecięcej, chirurgii onkologicznej, rehabilitacji lub rehabilitacji medycznej, lub rehabilitacji ogólnej, lub rehabilitacji w chorobach narządu ruchu. Wyżej wskazani lekarze-specjaliści mogą m.in. zlecać protezy kończyny dolnej i górnej. Natomiast ortozy kończyn dolnych i górnych mogą zlecać także lekarze posiadający specjalizację w dziedzinie neurologii i reumatologii. Niektóre wyroby medyczne może zlecać także lekarz podstawowej opieki zdrowotnej np. cewniki zewnętrzne, cewniki urologiczne,

zestawy infuzyjne do osobistej pompy insulinowej, worki stomijne samoprzylepne jednorazowego użycia, pieluchomajtki do 90 sztuk miesięcznie.

Zlecanie wyrobów medycznych w przypadku rehabilitacji związane jest z węższą dziedziną świadczeń zwaną tradycyjnie zaopatrzeniem ortopedycznym. Zaopatrzenie ortopedyczne definiowane jest w podręcznikach jako świadczenie zdrowotne mające na celu wyeliminowanie lub zmniejszenie dysfunkcji narządu ruchu poprzez dostosowanie choremu/pacjentowi odpowiednich przedmiotów ortopedycznych [12]. Obecnie fizjoterapeuta, jednakże nie każdy, może wystawiać zlecenia na określone wyroby medyczne. Zlecenia na wyroby medyczne po zmianie przepisów od 23 X 2019 r. może wystawiać magister (chodzi o fizjoterapeutę, o którym mowa w art. 4 ust. 4 pkt 1 i 2 ustawy z dnia 25 września 2015 r. o zawodzie fizjoterapeuty) i specjalista fizjoterapii. Przez specjalistę w dziedzinie fizjoterapii należy rozumieć fizjoterapeutę posiadającego tytuł specjalisty w dziedzinie fizjoterapii lub fizjoterapeutę posiadającego tytuł specjalisty rehabilitacji ruchowej II stopnia.

Przykładowo specjalista w dziedzinie fizjoterapii uzyskał prawo do wystawiania zleceń m. in. na: protezę uzupełniającą stopę, protezę kosmetyczną w obrębie ramienia, ortezę obejmującą gołę i stopę, ortezę obejmującą obręcz biodrową, uda, golenie i stopy, gorset odciażająco-stabilizujący, ortezę tułowia lędźwiowo-krzyżową, wózek inwalidzki ręczny, wózek inwalidzki specjalny (np. o napędzie elektrycznym). Natomiast fizjoterapeuta posiadający tytuł magistra fizjoterapii z prawem do samodzielnego udzielania świadczeń fizjoterapeutycznych ma uprawnienia do wystawiania zleceń m.in. na ortozy: stabilizujące staw skokowy, stawu kolanowego, na rękę i przedramię, stawu łokciowego, na ramię i bark [13]. Uprawniony fizjoterapeuta może przede wszystkim zlecać ortozy (aparaty ortopedyczne) i protezy, a także tzw. wyroby lokomocyjne nazywane też w piśmiennictwie pomocami ortopedycznymi specjalnymi lub przedmiotami pomocniczymi (laski, kule, wózki). Ortezę definiuje się jako techniczną konstrukcję podporową wykorzystującą zasady mechaniki klasycznej, stosowaną w celach leczniczych lub profilaktycznych, w szczególności u osób z chorobami narządu ruchu [14]. Protezy zastępują narządy z wrodzoną niepełnosprawnością, a także te, których pełna sprawność została utracona w wyniku np. wypadków lub chorób.

Zlecenie to dokument, którego wystawienie jest warunkiem uzyskania świadczenia i stanowi podstawę do rozliczenia udzielonych świadczeń. Zlecenia mogą być wystawiane po uprzednim osobistym badaniu fizykalnym świadczeniobiorcy lub badaniu za pośrednictwem systemów teleinformatycznych lub systemów łączności, a w przypadku zaopatrzenia w wyroby medyczne przysługujące comiesięcznie – także na podstawie dokumentacji medycznej świadczeniobiorcy [15]. Zlecenie na wyroby medyczne powinno zawierać konkretne informacje określone prawem. Aktualnie szczegółowe regulacje dotyczące treści zlecenia na wyroby medyczne zawiera rozporządzenie Ministra Zdrowia z dnia 2 sierpnia 2019 r. w sprawie

zlecenia na zaopatrzenie w wyroby medyczne oraz zlecenia naprawy wyrobu medycznego (Dz.U. 2019; poz.1555). Od roku 2020 weryfikacja zlecenia i potwierdzenie posiadania prawa do świadczeń opieki zdrowotnej, odbywają się za pośrednictwem serwisów internetowych lub usług informatycznych Funduszu.

DYSKUSJA I WNIOSKI

Polscy fizjoterapeuci po wielu latach starań doczekali się własnej ustawy – pragmatyki zawodowej. Dnia 25 września 2015 r. została uchwalona pierwsza w polskim systemie prawnym ustawa o zawodzie fizjoterapeuty. Ustawa o zawodzie fizjoterapeuty określa zakres i podstawowe standardy prawne (ustawowe) wykonywania zawodu fizjoterapeuty. Ich naruszenie stanowi podstawę do pociągnięcia fizjoterapeuty do odpowiedzialności prawnej zarówno cywilnej, jak i zawodowej, a także pracowniczej, jeżeli fizjoterapeuta ma status pracownika w rozumieniu kodeksu pracy. Ustawa powyższa zasadnie wprowadziła wymóg posiadania prawa wykonywania zawodu przez fizjoterapeutów udzielających świadczeń zdrowotnych. Ponadto ustawa ustanawia nowy, bardziej profesjonalny system kształcenia fizjoterapeutów obejmujący (po dniu 1 października 2017 r.) tylko jednolite 5-letnie studia magisterskie w zakresie fizjoterapii, 6-miesięczną praktykę zawodową oraz złożenie Państwowego Egzaminu Fizjoterapeutycznego (z wynikiem pozytywnym).

Po nowelizacji ustawy o zawodzie fizjoterapeuty i ustawy o działalności leczniczej dokonanej przez ustawę z dnia 9 listopada 2018 r. o zmianie ustawy o działalności leczniczej oraz niektórych innych ustaw (Dz. U. 2018; poz.2219) fizjoterapeuci mogą od 1 kwietnia 2019 roku, podobnie jak lekarze i pielęgniarki, zakładać praktyki zawodowe, a nie tylko podmioty lecznicze. Bowiem po wejściu w życie nowelizacji ustawy z 15 kwietnia 2011 r. o działalności leczniczej (art.5), działalność lecznicza fizjoterapeuty może być wykonywana w formie:

a. jednoosobowej działalności gospodarczej jako indywidualna praktyka fizjoterapeutyczna, indywidualna praktyka fizjoterapeutyczna wyłącznie w miejscu wezwania, indywidualna praktyka fizjoterapeutyczna wyłącznie w zakładzie leczniczym na podstawie umowy z podmiotem leczniczym prowadzącym ten zakład;

b. spółki cywilnej, spółki jawnej albo spółki partnerskiej jako grupowa praktyka fizjoterapeutyczna.

Po zmianie przepisów fizjoterapeuci, tak samo jak lekarze i pielęgniarki, mogą założyć, a potem prowadzić jeden z dwóch generalnych rodzajów praktyk zawodowych, tj.: praktykę indywidualną lub praktykę grupową.

Od 1 stycznia 2019 roku skierowanie na rehabilitację leczniczą ambulatoryjną oraz domową może w dalszym ciągu wystawić każdy lekarz ubezpieczenia zdrowotnego, jednakże zmieniła się obowiązkowa treść skierowania. Nie ma już bowiem obligatoryjnego zapisu w przepisach dotyczących świadczeń gwarantowanych w zakresie rehabilitacji leczniczej, który nakazywał, aby w skierowaniu określone były zlecone konkretne zabiegi wraz z liczbą poszczególnych

zabiegów w cyklu zabiegowym. Lekarz (poza specjalistą w zakresie rehabilitacji) obecnie nie decyduje już, jakie zabiegi fizjoterapii będą realizowane u jego pacjenta. Tym samym zostały ograniczone rola lekarza i jego zakres kompetencji w sferze rehabilitacji leczniczej. Jednakże w świetle stanowiska Prezesa NFZ zlecenie konkretnych zabiegów nie stanowi błędu w treści skierowania lekarskiego i nie stanowi o nieprawidłowości wystawionego skierowania [16].

W przypadku skierowania wystawionego przez lekarza ubezpieczenia zdrowotnego będącego jednocześnie specjalistą w dziedzinie: rehabilitacji ogólnej lub rehabilitacji medycznej, lub rehabilitacji w chorobach narządu ruchu zlecone na skierowaniu zabiegi, co do których fizjoterapeuta ma uzasadnioną wątpliwość, fizjoterapeuta może dokonać modyfikacji tych zabiegów po konsultacji z lekarzem kierującym. W przypadku pozostałych skierowań wystawionych przez innych lekarzy ubezpieczenia zdrowotnego, będących jednocześnie specjalistami w innych dziedzinach medycznych również tych realizujących świadczenia w ramach poradni lekarskiej rehabilitacyjnej, zlecone zabiegi stanowią jedynie sugestię lekarza co do planu terapii i nie są wiążące dla fizjoterapeuty.

Podkreślić należy, że zlecać konkretne zabiegi fizjoterapii może nie każdy fizjoterapeuta, a tylko uprawniony do tego (tj. posiadający odpowiednie wykształcenie/staż pracy). W praktyce fizjoterapeuta, który otrzyma skierowanie z wpisanymi przez lekarza zabiegami może:

- a. nie zmieniać zleconych zabiegów (czyli zrealizować wszystkie zabiegi wpisane przez lekarza);
- b. zmienić część zleconych zabiegów (czyli zrealizować tylko niektóre zabiegi wpisane przez lekarza oraz inne zlecone samodzielnie);
- c. ustalić wszystkie nowe zabiegi (w całości samodzielne zlecenie fizjoterapeuty).

Zmiany w organizacji udzielania świadczeń gwarantowanych z rehabilitacji leczniczej wynikają przede wszystkim z ustawowego zwiększenia kompetencji fizjoterapeutów i tym samym wzmacniają ich rolę w systemie ochrony zdrowia. Nowe prawo fizjoterapeutów do samodzielnego zlecenia określonych wyrobów medycznych wpływa także na wzrost ich roli/wzmocnienie pozycji w systemie ochrony zdrowia. Jednakże w przypadku zlecenia wyrobów medycznych lekarze nie stracili dotychczasowych uprawnień. Powyższe uprawnienie fizjoterapeutów można z jednej strony uznać za korzystne dla lekarzy – odciąża ich bowiem w pracy, z drugiej zaś może pozbawić ich wpływu na cały proces leczenia pacjenta (w szczególności ortopedów).

Bezspornie nowe przepisy dotyczące fizjoterapeutów i rehabilitacji leczniczej to nie tylko prawa, ale i większe obowiązki oraz większa odpowiedzialność fizjoterapeutów. Przedmiot sporu środowiskowego (z lekarzami) i kontrowersje dotyczą przede wszystkim zakresu czynności, do jakich samodzielnie uprawniony jest fizjoterapeuta (samodzielnego kwalifikowania pacjentów do zabiegów).

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REVIEW ARTICLE

AN INTERDISCIPLINARY APPROACH TO THE MANAGEMENT OF CRITICALLY ILL PATIENTS DURING COVID-19 PANDEMIC; AN EXPERIENCE OF A UNIVERSITY HOSPITAL IN ENGLAND

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ABSTRACT

COVID-19 pandemic presents significant challenges in delivering safe and efficient patient care, especially during the surges. In all health care systems, provision of available critical care facilities is a scarce resource, even in normal times. Problematic is not just the limitation of physical spaces in intensive care units, but also the availability of trained personnel. The critical care model, developed in Queen Elizabeth Hospital Birmingham to cope with the surge of COVID-19 patients, is based on early implementation of an interdisciplinary approach and extensive cooperation between the branches of practice, allowing to address both challenges. The main pillars are early upskilling of non-critical care staff, creation of safe, streamlined clinical pathways, adjustment of the physical layout of critical care units and comprehensive cross-town cooperation allowing to accommodate an increased number of patients, requiring intensive care. The model was well tested in clinical practice, enabling the hospital to increase the critical care footprint by more than 200% during the pandemic's surge between March and May 2020.

KEY WORDS: Critical care, clinical governance, interdisciplinary intensive therapy, COVID-19, medical education, pandemic

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INTRODUCTION

COVID-19 disease initially originated in Wuhan, Hubei Province in the People Republic in China, and very quickly spread across the world, reaching the United Kingdom on the 31st of January 2020. On the 11th of March, the World Health Organization (WHO) declared COVID-19 as a worldwide pandemic. SARS-CoV-2 virus has been identified as a cause of the disease. Even before that announcement, on the 3rd of March Department of Health and Social Care of British Government, published Coronavirus (COVID-19) action plan outlining what the health and social care system across the UK had done to tackle the coronavirus (COVID-19) outbreak and the plans for the next steps [1].

National Health Service (NHS) England, the overarching regulatory body, instructed all Hospitals to prepare for the impending surge of patients [2]. The initial set of guidelines was issued on the 17th of March 2020, providing a framework allowing for the rapid expansion of critical care services using a multidisciplinary approach [3]. The national priorities for England were designed to increase Critical Care capacity at least threefold, allowing for the provision of safe and efficient care not only for Covid-19 positive patients but also allowing for a continuous inflow of non-COVID emergencies and from health and safety point of view a provision of the safe working environment for medical staff [4].

The provision of critical care beds is a scarce resource across all countries with the United Kingdom and Poland having

a very similar number of intensive care beds per 100 000 inhabitants – 6.6 and 6.9 respectively [5]. Our healthcare organisation – University Hospitals Birmingham NHS Foundation Trust consists of four hospitals providing secondary and tertiary care for the West Midlands region, the second most populated metropolitan area in England, with very high population density and considerable areas of social deprivation. Jointly the total provision of critical care beds across all four sites is around 110, with more than 70 beds alone available at the biggest site – Queen Elizabeth Hospital Birmingham [5].

THE AIM

The national and local expectations were that significant reconfiguration of health care provision, especially critical care, need to be made to face the challenge of COVID-19 surge. Therefore, the aim of the paper is to share our experience of organizational challenges and adjustments required in the midst of pandemic.

RESPONSE TO CHALLENGES

Based on the guidelines, published jointly by the specialist learned societies and colleges, the multimodal approach was used to prepare for the inevitable surge of the patients [6]. From the onset, it became apparent that if the surge of the patients required a two or threefold increase of critical

care capacity, it would be impossible to continue with business as usual. An innovative way of working was needed to be established. It had also become clear that to ensure continuity of care and improve the resilience of medical staff, 12 hours resident shift ought to be introduced in place of the standard on-call arrangements. The predicted, massive surge of patients, on the one hand, precluded the division of hospitals into hot and clean sites. On the other hand, as in Poland, the model required to establish ways to continue the provision of urgent and emergency secondary and tertiary care at all sites.

The special emphasis was put on the following pathways to achieve organisational aims:

- creation of control and command centres, with regular meetings and clear daily communication at Trust, hospital and departmental levels, utilising modern media channels;
- development of critical care workforce by quick upskilling medical professionals with previous exposure and experience of the intensive care medicine to allow redeployment of staff to more occupied areas;
- increase in critical care physical capacity across all four sites, enabling to accommodate the surge of critically ill patients and safe transfers between the hospitals of the organisation;
- introduction of dedicated teams, allowing to streamline very complex, albeit repetitive procedures such as rapid intubations of clinically deteriorating patients or proning ventilated patients;
- standardisation of medical and nursing guidelines, allowing from human factors perspective to provide reliable and consistent clinical care, despite constant rotation of medical and nursing staff;
- simplification of medicinal governance, with special emphasis on premade infusions and prefilled syringes;
- provision of personal protection in accordance with Public Health England (PHE) guidelines;
- a widespread introduction of pastoral care for medical and nursing staff allowing for easy access to wellbeing and restorative practice teams.

The above-mentioned goals were achieved in several different, interconnected and complementary ways.

Creation of control and command centres with different, but clear responsibilities allowed for constant two-way communication. The responsiveness was increased by the continuous in-hospital presence of senior management and clinicians, taking part in twelve-hour shifts. It was especially crucial when the different districts of Birmingham surged sequentially, and fast decisions on patients and personnel transfers between critical care units of our organisations were required to ensure resilience and to balance the burden of the admissions between the hospitals. To comply with social distancing rules, and reduce potential exposure, modern technologies were widely implemented, inclusive of video conferencing and messaging platforms.

The immediate (approximately four weeks before anticipated peak of COVID-19-related admissions) closure of non-emergency operating rooms, outpatient clinics, and day-case units

allowed to significantly increase the available medical and nursing workforce. The massive upskilling program consisted of the provision of more than 20 standardised educational lectures and hands-on sessions, increasing critical care medical staffing by more than 200 doctors, fully compliant with national directives [6, 7]. Initially, the focus was on non-critical care operating rooms (OR) staff, later on expanding to many other branches of practice. Doctors from a wide range of medical and surgical specialities were upskilled, inclusive such disciplines as neurology, ophthalmology or plastic surgery.

Closure of operating theatres also allowed to move anaesthetic equipment, such as ventilators, infusion pumps, and monitors to critical care as well as spare hospital beds. The physical constraints of limited space in the critical care unit were overcome by having two beds in one cubicle. This alone required detailed planning by the estates and medical engineering to ensure a sufficient supply of medical gases and fluids. The temporary layout of the ICU, although very crowded, was fully functional. The physical dividing walls were erected to separate clean areas from COVID ICU, as fully operational non-COVID ICU was maintained throughout the pandemic.

Closure of elective activities in the hospital put a strain on emergency workflow, especially in the supra-regional surgical specialities. However, highly sub-specialised procedures requiring emergency input (such as neurosurgery, burns, cardiothoracic surgery and hyperacute solid organ transplantations) were still successfully provided within the four hospitals of the Trust. The capacity in the private hospital providers, as well as NHS hospitals routinely serving non-urgent cases, was used to ensure delivery of urgent operations throughout the surge.

Within COVID designated ICU areas, streamlining of complex medical procedures allowed to deliver high quality, standardised medical management to the patient, even when the footprint in intensive care exceeded 200% pre-COVID capacity. The key factor was the utilisation of transferable skills of many medical professionals, not routinely involved in critical care.

For example, the insertion of central lines was provided by interventional radiologists, proning of ventilated patients by neurosurgeons, and OR staff, and bedside tracheostomies by ENT (otolaryngologists) and maxillofacial surgeons. To allow consistency, checklists were employed widely, and allied health professionals such as physiotherapists were empowered to enforce them. Not surprisingly, this led to improved compliance with pre-existing medical guidelines, such as on lung-protective ventilation. An added benefit from having a multidisciplinary team approach was the availability of instant speciality input into patient displaying a plethora of multiorgan complications related to COVID-19.

Medicinal governance was focused on minimising possibilities of inadvertent medical errors. This was achieved by standardisation and simplification of the prescriptions. Hospital pharmacy was involved in the preparation of premixed infusions of inotropes and sedatives to minimise workload at the bed spaces [8]. Wherever there was an alternative medication allowing for less frequent or less

burdensome administration, the change of prescription was sought. In case of any significant changes, immediate communication via control and command channels followed, allowing for a relatively quick change of standard practice. If a deviation from previous practice was deemed necessary, the change in the electronic prescription system utilised by Trust (Birmingham Systems Prescribing Information and Communications System - PICS, Birmingham, UK) was immediately implemented. One of the examples could be the increase in the dose of anticoagulants, namely enoxaparin in COVID patients in line with emerging national and international recommendations [9].

The provision of appropriate personal protection equipment (PPE) was based on Public Health England (PHE) guidelines [10]. The emphasis was not only on the sufficient supply of correct PPE to health care workers but also on making sure that PPE is applied and disposed of in each and every case in the correct way, reducing the risk of transmission and contaminations especially during the aerosol-generating procedures and particularly at the doffing [11]. This was achieved in two separate, but interconnected ways.

Outside critical care, highly specialised assessment teams, led by senior clinicians, were available around the clock to provide a clinical review of suspected patients with COVID. If the patient was pre-qualified for admission to critical care, fully donned in PPE team of the senior anesthesiologists was deployed to secure the airways and following endotracheal intubation to transfer and establish mechanical ventilation in critical care. Such an approach allowed us to minimise the risk of contamination during initial aerosol-generating procedures [12].

Inside ICU, the donning and doffing stations were established. All stations were staffed by nominated officers, with the responsibility of advising on and enforcing the predefined sequence on donning and doffing of PPE. The officers had the authority to stop anyone not following the sequence, as well as to limit flow through the stations to minimise the possibility of cross-contamination and subsequent infection. The efficacy of such an approach was further assessed by our Trust. SARS-CoV2 seroconversion rate between critical care workers in UHB was only 14.8%; hence it was lower than in the tested overall population of health care workers in our Hospitals, in whom it has reached 24.4%. It may be interpreted, albeit with caution, that health and safety approach, described in detail above, allowed to minimise occupational risk, even with the significant exposure to the viral load [4].

The special emphasis was put on staff wellbeing and restorative practice, to minimise well-recognised psychological trauma caused by the pandemic [13]. The level of stress was increased as many of staff members were working outside their normal practice, with an obvious initial poor understanding of COVID-19 pandemic, especially in view of inevitable bad outcomes. Support was provided by a previously established wellbeing group consisting of senior clinicians and restorative practice nurses. In addition, quiet rooms were created to allow for rest and mindfulness, away from clinical areas. At the end of the surge, all redeployed medics were offered feedback sessions, allowing for de-

briefing and further discussion.

DISCUSSION

Implementing changes in organisation of the care, fast-tracked upskilling programs for staff across the board, with collaborative work of Trust employers outside they normal practice, guided by pre-written checklists with clear escalation paths, allowed our Hospital and the Trust to fully function throughout the surge of COVID-19 patients from March until mid-May 2020.

The number of patients admitted to the ICU in QEHB has been similar to the number of patients reported in the most affected critical care units in other nations, such as in Milan (Italy) or in New York (USA) [14, 15]. In total, the intensive care so far was provided to more than 180 patients tested positive for SARS-CoV-2. The overwhelming majority required mechanical ventilation, with an average length of stay in critical care of 19 days. The survival rate of closed cases (patients who either were discharged alive or died on the unit) is around 67% which is in line with case-mix data provided nationally by the Intensive Care National Audit & Research Centre (ICNARC) [16] detailed results of medical management for the Trust and the region are presented further in ICNARC publications, hence not analysed in detail in this correspondence (16).

CONCLUSIONS

The achieved clinical outcomes support the efficacy of the multimodal plan implemented before the surge. A relatively low seroconversion rate between critical care staff outlines the safety of the departmental approach to personal protection. The positive feedback from doctors redeployed to critical care during the pandemic shows the importance of team model and joint decision making in medical practice.

In summary, we believe that a multidisciplinary approach, used in our organisation, allowed for the provision of safe and efficient critical care throughout the surge of critically ill patients during COVID-19 pandemic.

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CASE STUDY

CONSERVATIVE TREATMENT AS A THERAPEUTIC OPTION FOR PENETRATING AORTIC ULCER OF THE AORTIC ARCH IN A PATIENT WITH BICUSPID AORTIC VALVE

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Penetrating aortic ulcer (PAU) is ulceration of an aortic atherosclerotic plaque penetrating through the internal lamina into the media. PAU is a rare condition and occurs in 2% – 7% of acute aortic syndromes (AAS); however, the actual incidence is unknown because of asymptomatic patients. One may treat it conservatively as well as surgically. We present a case of a 54-year-old man, who was admitted to hospital due to the exaggeration of exertional chest pain and persistent headaches. During coronary angiography, the suspicion of PAU was raised. Contrast-enhanced computed tomography confirmed the diagnosis. Transesophageal echocardiography showed bicuspid aortic valve with minimal calcification, the dilated ascending aorta, large atherosclerotic plaques in the aortic arch with ulceration (thickness: 5.0 – 5.5 mm, diameter: 5 – 6 mm, depth: 3 – 4 mm), without intramural hematoma. Conservative treatment was chosen with uneventful 2-year follow-up. Although surgical management is advocated for patients with PAU type A, we demonstrated that type A PAU can be successfully treated conservatively as well.

KEY WORDS: acute aortic syndrome; PAU; stanford type A; treatment options; aortic diseases

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INTRODUCTION

Penetrating aortic ulcer (PAU) is ulceration of an aortic atherosclerotic plaque penetrating through the internal lamina into the media. PAU is a rare condition and occurs in 2% – 7% of acute aortic syndromes (AAS); however, the actual incidence is unknown because of asymptomatic patients [1]. Only about 18% of all cases are symptomatic [2]. Due to location PAU may be divided into type A – starting in the ascending aorta and type B – in the descending aorta [2]. Ulcers in the aortic arch are found rarely, and more often than in the descending aorta, they are associated with acute complications [1].

Treatment of PAU may be conservative and/or interventional. The level of evidence in treatment is low due to the lack of randomized trials [3]. The structure of the aortic arch is complicated because of the presence of three crucial branches. Moreover, patients with ulcers often suffer from comorbidities; therefore, the question is whether the intervention is always justified. We present a case of a patient with type A PAU successfully treated conservatively.

CASE REPORT

A 56-year-old male patient with a history of arterial hypertension and tobacco-smoking was admitted to our department due to the intensification of typical exertional

chest pain for a couple of weeks. On admission, he was stable and complained about persistent headaches.

Physical examination revealed elevated blood pressure (BP, 170/100 mmHg), and a systolic heart murmur in the right second intercostal space radiating towards carotid arteries. Three-months earlier, transthoracic echocardiography (TTE) showed left ventricle ejection fraction (LVEF) of 65%, left ventricular diastolic diameter (LVDd) of 55 mm, left atrium (LAD) of 47 mm. Also, the suspicion of the bicuspid aortic valve (BAV) was raised. Additionally, aortic dilatation was observed with the aortic bulb (AoB) of 44 mm, the aortic arch of 40 mm, the middle part of the aorta of 32 mm, and the dilated descending aorta (AoD). The patient was being diagnosed in the direction of arterial hypertension and ischemic heart disease. At discharge, a patient received atorvastatin, acetylsalicylic acid, ramipril, and amlodipine. Planned coronary angiography took place three months later and showed non-obstructive coronary arteries (Fig. 1 A – B). During the procedure, the operator raised the suspicion of “something” in the aorta. Further computed tomography (CT) showed ulceration near the brachiocephalic trunk within atherosclerotic plaque (Fig. 2 A – B) consistent with diagnosis of type A PAU. It also showed the following: AoB 46 mm, AoA 45 mm, AoD 28 mm, the aortic arch 37 mm and BAV was confirmed. After treatment modification, the patient had satisfactory

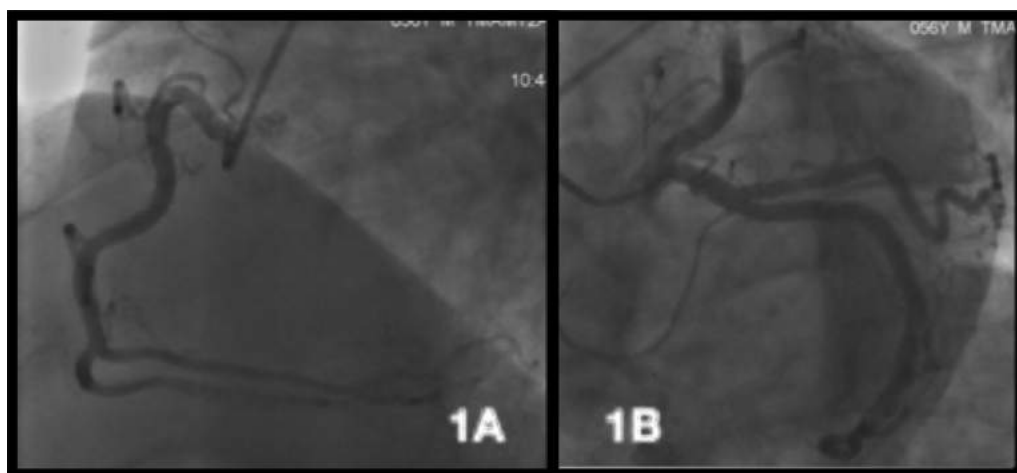


Fig. 1. Coronary angiography showing non-obstructive coronary arteries. A: Right coronary artery, B: Left coronary artery.

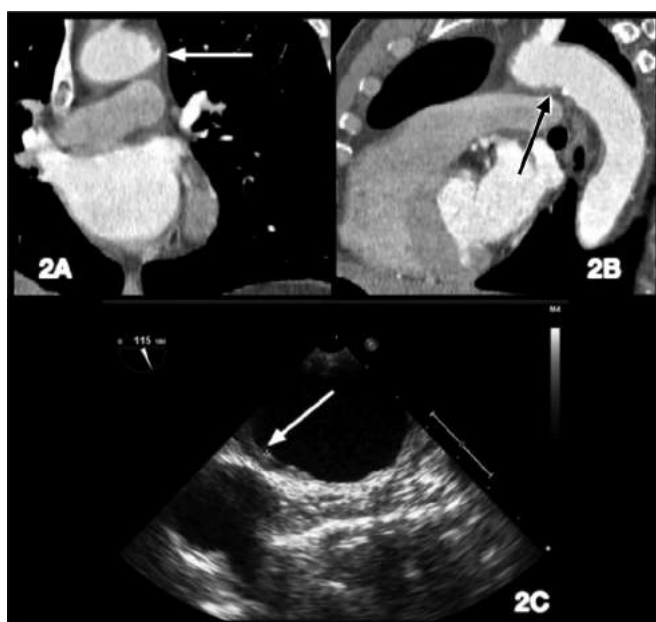


Fig. 2. Penetrating aortic ulcer in different imaging modalities. A – B: Cardiac computed tomography in different views showing the lesion below brachiocephalic trunk; C: Transesophageal echocardiography.

BP control and remained chest pain-free. At the Heart Team the decision about conservative treatment was made, and bisoprolol was added to the regimen.

Two months later, transesophageal echocardiography (TEE) showed BAV with minimal calcification, the dilated ascending aorta, large atherosclerotic plaques in the aortic arch with ulceration (thickness: 5.0 – 5.5 mm, diameter: 5 – 6 mm, depth: 3 – 4 mm), without intramural hematoma or penetration (Fig. 2 C). Conservative treatment was continued.

After one year, TTE showed LVEF 65%, LVDd 48 mm, AoA 46 mm, BAV, AoB 45 mm, the aortic arch of 36 mm, proximal segment of AoD 29 mm. In comparison with previous examinations valves function improved. Moreover, TEE revealed: AoA as in previous examination, large plaques in AoD, in aortic arch a smaller atherosclerotic plaque with ulceration (diameter: 3 mm, depth: 2.5 mm). The CT results were similar to the previous study.

After two years, TTE results were comparable with previous examination, and CT findings showed new thrombi around previous ulceration, AoB 46 mm, AoA 43 mm, aortic arch 37 mm, and AoD 28 mm. Again, the decision about conservative treatment was made.

DISCUSSION

Our patient was initially diagnosed with arterial hypertension. It is difficult to say whether the pain at admission was caused by the exacerbation of the comorbid disease or PAU. Differentiation of PAU from other AAS and other cardiovascular diseases, based only on clinical symptoms, may be difficult due to non-specific symptoms; therefore, radiological diagnostics is very useful and reveals a characteristic picture. In making the diagnosis of PAU contrast-enhanced CT remains the method of choice [3]. During CT scans, it is essential to use contrast because otherwise, PAU may be mistaken for intramural hematoma (IMH) [3].

Our patient had only arterial hypertension; however, patients with PAU are usually older (>70 years). Other common comorbidities, not present in our case, are ischemic heart disease, chronic obstructive pulmonary disease, renal insufficiency, and coexisting abdominal aneurysm [3, 4].

The natural course of PAU is a matter of debate. The ulcer may remain stable or may lead to the development of saccular or fusiform aneurysms, aortic enlargement, transmural rupture, and formation of medial hematoma [3, 4]. The main goal of PAU treatment is to prevent aortic rupture and progression to acute aortic dissection. The risk of aortic rupture for symptomatic PAU may be up to 40%, even without the enlarged aortic diameter [1, 5].

European Society of Cardiology (ESC) guidelines from 2014 recommend conservative treatment with analgesic therapy and BP control is recommended in all patients with PAU [3]. The most commonly used antihypertensives are beta-blockers and calcium channel blockers. Pain control is also vital to limit catecholamine burst and subsequent tachycardia and BP increase [1]. Target values for BP and heart rate are 100-120 mmHg and 60-80 bpm, respectively, and are the same as in other AAS [6].

The further strategy depends on the type of lesion. In type A symptomatic cases, due to more frequent complications, patients should undergo urgent surgery [1, 3]. In type B cases, it is recommended to introduce conservative treatment under careful observation. Among patients with uncomplicated type B PAU, imaging surveillance is suggested. In complicated cases, patients should have interventional therapy considered. There are two therapeutic options in this case: thoracic endovascular aortic repair (TEVAR) and open surgery. However, because of elderly age and comorbidities open surgery is often not recommended. TEVAR seems to be a better option for these patients and is more preferred than surgery by ESC (class of recommendation IIa vs. IIb, respectively) [3]. TEVAR is mainly used to treat the descending aorta. Endovascular treatment of ulcers localized in the aortic arch is more difficult and complicated. However there are more and more techniques available for treating ulcers in the aortic arch, where endografts with branches and fenestrations are used [7].

Our patient had no predictors of the disease progression like refractory and recurrent pain, increase in pleural effusion and PAU diameter > 20 mm and PAU neck > 10 mm. These risk factors are useful to determine the need for interventional treatment [2, 3, 6]. Not all studies confirmed the validity of the PAU dimension criterium [1].

In our case, pharmacology treatment introduced at the beginning was not focused at PAU. We managed to achieve proper BP values and relieve pain. After treatment modification, the patient's response was satisfactory. Moreover, our patient had no negative prognostic factors, which would indicate an urgent need for surgery. By the used treatment, it was possible not only to stop the development of the disease but also to reduce the size of the ulcer by almost 50% after two years.

CONCLUSIONS

In making a diagnosis of PAU contrast-enhanced CT remains the method of choice. However, in presented case, coronary angiography with aortography appeared to be crucial to raise the suspicion of PAU. In type A PAU, patients are usually treated surgically unless intractable comorbidities are present. Although surgical management is advocated for patients with PAU type A, we demonstrated that type A PAU can be successfully treated conservatively as well.

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CASE STUDY

HYDROXYCHLOROQUINE IS NO MIRACLE CURE FOR COVID-19 INFECTION –IMAGING AND CLINICAL COURSE IN AN ELDERLY FEMALE

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The risk for an unfavourable course of SARS-CoV-2 pneumonia rises with age and comorbidities. We report the case of an elderly female where the sum of such factors – together with massive findings in the computed tomography of the lung – led us to a therapy with hydroxychloroquine as a compassionate use. The unfavourable outcome demonstrates that – despite the enthusiasm of some authors – hydroxychloroquine is no miracle drug. The worldwide SOLIDARITY trial will help clinicians to assess the potential of the repurposed antimalarial drugs better.

KEY WORDS: chest CT, Chloroquine, compassionate use, COVID-19, Hydroxychloroquine, repurposed agent, SARS-CoV-2

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INTRODUCTION AND THE AIM

Ai et al. [1] recently published an imaging study in 1014 patients who had chest computed tomographies (CT) performed and reported a sensitivity of 97% for the SARS-CoV-2 virus pneumonia. That said, computed tomography of the thorax has become an important tool not only in the detection of SARS-CoV-2 pneumonia, but also for an evaluation of the severity of the pulmonary involvement [2]. Advantages of an early CT imaging (if available) are a higher sensitivity to detecting lung opacities compared to chest radiographs where the lack of visible early abnormalities may cause a higher number of false-negative patients. The chest CT reveals typical radiological features such as ground-glass opacities, multifocal patchy consolidations and interstitial changes with a peripheral distribution. Furthermore, COVID-19 testing may take about a day and can be problematic due to limited laboratory capacities and lack of nucleic acid kits [2].

If a massive finding in the chest CT is noted, the clinician is faced with the problem of not having a specific antiviral therapy available. This is a problem that is exacerbated if the patient's prospects for the further course of the disease are already poor, usually due to age and comorbidities. Although multiple drugs with potential against COVID-19 are still in the experimental stages in development, according to the current status only the "repurposed agents" hydroxychloroquine (HCQ)/chloroquine and remdesivir, as well as lopinavir/ritonavir can be considered as options. Remdesivir was originally developed in therapy against the Ebola virus, but showed no significant advantages here [3], with regard to its use against the COVID-19, there are yet only published data from a compassionate use study available [4]. Robust and significant results from randomized clinical trials are

still pending. This also applies to hydroxychloroquine/chloroquine, which are actually used as medication for malaria. Compared to remdesivir, these preparations have the advantage of immediate availability and low costs. They are also recommended for use in China, for example by the multicenter collaboration group of the Department of Science and Technology of Guangdong Province and Health Commission of the Guangdong Province [5]

CASE DESCRIPTION

In April 6th, a 88-year-old female was admitted to our clinic due to increasing dyspnea. She lived in a nursing home for the elderly where some residents including her had already been tested positive for COVID-19 infection. Her comorbidities were hypertension, atrial fibrillation and she suffered from hemiparesis of the right body half after a hemorrhagic stroke in 2009. Upon hospitalization, the patient was fully alert and vigilant, serum procalcitonin was negative and serum C-reactive protein was 109 mg/l (<5).

Further laboratory values at admission:

Pathological: ASAT 1.44 μ kat / l (0.17-0.85); d-dimer 4166 (<230 μ g/l); creatinine 156 (44-80 μ mol/l). In the small blood count with an otherwise normal status and normal leukocyte count, the lymphocytes were found to be slightly reduced in absolute terms at 0.4 Gpt / l (1.1-3). The following values were within normal range: sodium, potassium, ASAT, gamma -GT, troponin-T, coagulation, TSH, fT3, fT4.

The CT of the chest showed extensive bipulmonary ground glass opacities with a major preponderance in the lower lobes of the lung, typical for a COVID-19 infection (Fig. 1-3). Since the extent of the infiltrations made an unfavourable course

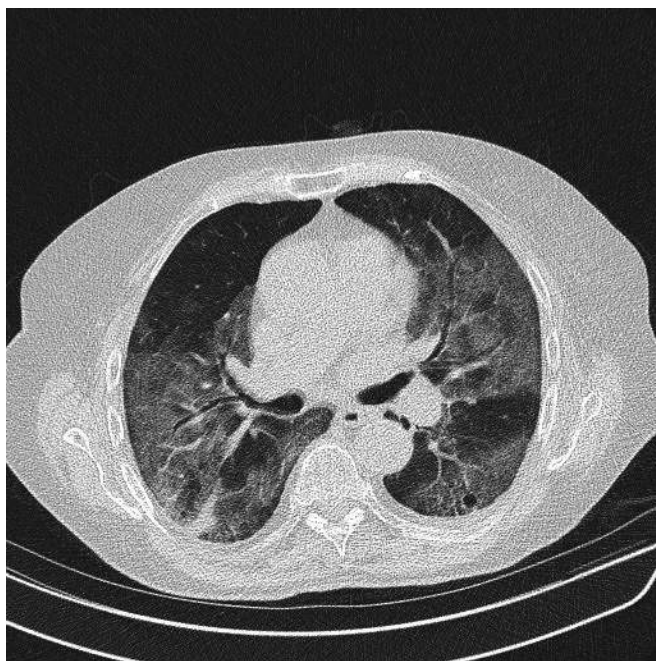


Fig. 1. Axial reconstruction with slice thickness and interval of 1.5 mm. This slice shows multiple ground-glass opacities with involvement of the upper and lower lobes as well as a subpleural dominant reticular pattern and an early consolidation in the right lower lobe.



Fig. 2. Sagittal reconstruction with slice thickness and interval of 3.0 mm. This view demonstrates the dorsally predominant distribution of the ground-glass opacities and the involvement of the posterior part of the right middle lobe. In particular the small interlobar effusion, which is uncommon for Covid-19-pneumonia and probably caused by the comorbidities, is to be noted.

likely and after obtaining the patient's as well as her daughter's informed consent, a therapy with hydroxychloroquine (400 mg bid.) was immediately started as compassionate use. The dose was reduced to 200 mg bid. after two days and



Fig. 3. Coronal reconstruction with slice thickness and interval of 3.0 mm. This image demonstrates the extension of the ground-glass opacities and the grazing-paving pattern in the posterior segments of the right and left upper and lower lobes.

maintained. The daily electrocardiogram (ECG) monitoring revealed no ECG changes suggestive for cardiotoxicity and the patient had no gastrointestinal side effects. However, especially in terms of respiratory problems, the clinical course was unstable with highs and lows in the patient's condition. Finally, her clinical condition deteriorated and she died on April 11th due to cardiopulmonary failure.

DISCUSSION

Chloroquine, and its related derivative HCQ, are drugs that are commonly used for treating malaria. They have gained attention within the medical community as a potential therapy for the SARS-Cov-2 pneumonia as repurposed agents. Several studies worldwide (at least 15 in China) are still investigating the efficacy and safety profile of these drugs and in preliminary results, chloroquine was reported to be superior to control therapy by improving lung image findings, promoting the virus-negative conversion and shortening the disease course [6]. Some of these effects may be based on an interference with ACE-2-receptor mediated endocytosis [7].

Despite the enthusiasm of some authors [6], the WHO states that currently, there is still an insufficient database to assess the efficacy of chloroquine and HCQ in treating patients with COVID-19, or in preventing them from contracting the coronavirus [8]. As for the more commonly used HCQ, it needs to be considered, that it can lead to QT interval prolongation and torsades de pointes arrhythmia in susceptible individuals. Furthermore, co-prescription of other drugs such as azithromycin (which is also sometimes used for the treatment of COVID-19) could amplify this risk. HCQ also interacts with other cardiac drugs such as beta blockers and digoxin and increases the blood levels of these drugs [9].

In the case described here, the considerable amount of pulmonary infiltrations, together with the age and the comorbidities of the patient made a fatal course of the disease very likely. This is also underlined by the elevated levels of d-dimer since elevated levels of d-dimer are discussed as a marker for an unfavorable outcome in SARS-CoV-2 infections. Zhang et al. [10] reported significantly higher levels of d-dimer as associated with a more severe course of the disease in an analysis of the records of 140 hospitalized COVID-19 patients. This sum of factors is why we had decided for an immediate compassionate use of HCQ. However, the case exemplifies that a therapy with HCQ is far from a miracle cure. To date, a number of clinical trials are in progress to test the safety and effectiveness of other candidate drugs such as Interferon- α , lopinavir/ritonavir, ribavirin or arbidol [11]. However, the high number of studies (at least 300 currently) [12] on such drugs could unfortunately also lead to positive effects being observed in various (small) subgroups, with the situation becoming increasingly confusing. Fortunately, the World Health Organization (WHO) has organized a multinational randomized trial (SOLIDARITY trial) to study the effect of drugs that have been identified as promising based on in-vitro data and clinical experience with SARS-CoV-2 infections: These are remdesivir, lopinavir and ritonavir; furthermore the combinations lopinavir and ritonavir + interferon; and chloroquine or hydroxychloroquine [13].

CONCLUSION

In the age of the COVID-19 pandemic, the compassionate use of some drugs – especially in severe cases with poor prognosis – can be justifiable. Since the use should then naturally take place quickly, chloroquine or HCQ are suitable in most countries due to the rapid availability. This therapy is already recommended by some specialist societies – especially in Asia. But it is by no means a miracle medication, as the case described here shows. The current discussion is also increasingly focusing on the cardiotoxic potential of antimalarials. Hopefully, the WHO SOLIDARITY study will soon provide clinicians with reliable and robust information about the true value of these drugs.

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