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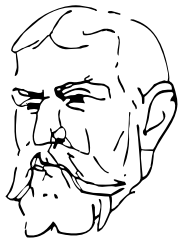

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ORIGINAL ARTICLE

USING THE SODA SCALE TO ASSESS THE EFFECTIVENESS OF NEUROLOGICAL SPEECH AND LANGUAGE THERAPY ON IMPROVING LANGUAGE FUNCTIONS IN POST-STROKE PATIENTS

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ABSTRACT

The aim: Assess the impact of neurological SLA on improvement of language functions in post-stroke patients.

Materials and methods: The study covered a total of 82 patients aged 40-80, of both sexes, with stroke-induced cognitive deficits. They were in-patients of the Clinic Of Rehabilitation And Physical Medicine of the "WAM" Teaching Hospital in Łódź. The study comprised two groups. The study group consisted of post-stroke patients with aphasia, who were referred to four-week comprehensive program of neurological SLA therapy earlier (about a month) after experiencing stroke. The group comprised 44 patients. Six months after the stroke, the patients underwent another four weeks of neurological SLA. The control group consisted of post-stroke patients with aphasia. The group comprised 38 patients. The four-week neurological SLA therapy was applied more than six months after stroke ("late therapy"). The patients were ascribed to the groups randomly. Language abilities were evaluated with the Aphasia Dynamics Assessment Scale (Polish: Skala Oceny Dynamiki Afazji, abbrev. SODA).

Results: The study showed that neurological SLA helped patients in both the study and control group to significantly regain their language functions. However, greater improvement was found in the study group, which underwent neurological SLA twice, which proves that duration of the therapeutic process is an important factor.

Conclusions: neurological SLA was beneficial for both groups, regardless of the time that elapsed between stroke and start of the therapy. neurological SLA therapy allowed patients to restore part of their language functions, both in the study and control group. The sooner neurological SLA is implemented and the longer its duration, the more beneficial it is to patients.

KEY WORDS: stroke, aphasia, speech and language therapy, neurological SLA

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INTRODUCTION

Besides motor deficits, the clinical features of stroke includes cognitive, language and emotional dysfunctions. They hinder the patient's recovery to optimal functioning in everyday life. This kind of brain damage is a tremendous shock to the sufferer. One is faced with both physical and mental disabilities, and the world known so far is falling apart.

A patient suffering from aphasia shows impairments in areas such as cognition, language, emotions and motivation. Patients are unable to express views, information or requests, they cannot define their needs, name emotions or feelings. This leads to anxiety and a feeling of powerlessness, often depression. Inability to return to everyday activities, work or to communicate with relatives, difficulties in reading and writing, problems with handling matters which were previously simple – such experiences challenge post-stroke patients both physically, mentally, and intellectually.

The data of the Department of Analysis and Strategy of the National Health Fund (October 2019) show that the scale of the problem is enormous. In Poland, about 70,000 people suffer a stroke each year. Of these, 90% are patients with risk factors. Those risks include heart diseases, inadequate diet, obesity, addictions, diabetes, as well as chronic stress. Post-stroke patients often require not only months of treatment, but also years-long, sometimes even lifelong, rehabilitation. However, it is important to note that post-stroke disability is not irreversible [1].

Left-hemisphere stroke patients often suffer from aphasia. There are chances for recovering language and cognitive functions with speech and language therapy.

Aphasia is a speech disorder that manifests itself as partial or complete loss of the ability to build and understand speech and written texts. This is caused by focal damage of varying extent to the speech area of the dominant brain hemisphere [2].

According to the World Health Organization, aphasia is not only an *impairment* consisting in the reduced ability to use language and speech. It is primarily a disorder of the ability to communicate causing *disability* [3]. Communication is executed primarily with language, so its deficits considerably disrupt a patient's professional, family, and social life. Because aphasia is defined as an acquired disorder or loss of language abilities resulting from organic brain damage, it is worth considering whether aphasia is rightly being called a *disease* [4]. From the point of view of

neurology, it is one of the symptoms of focal brain damage. Because it is a symptom, it is therefore not a disease but its effect. Undoubtedly, however, aphasia is rightly considered an important subject of diagnosis, because its etiology cannot be determined without detailed diagnostic tests, which in turn determine proper therapy. [5]

Aphasia is without doubt a result of universal and individual factors. Therefore, its severity in a given patient depends on the factor that caused it. The degree of disability correlates with the patient's ability to compensate its effects, which may be acquired in the course of therapy. The degree to which aphasia may be limited depends on to the extent the patient will be able to adapt to the new situation [6, 7].

The aim of the study was to evaluate of the impact of the speech and language therapy on improvement of language functions in post-stroke patients.

THE AIM

Assess the impact of neurological SLA on improvement of language functions in post-stroke patients.

MATERIALS AND METHODS

82 patients aged 40-80, of both sexes, with post-stroke cognitive deficits participated in the study. They were patients of the Rehabilitation and Physical Medicine Clinic of the University Teaching Hospital of the WAM Teaching Hospital in Łódź. The subjects were selected after considering the study's inclusion and exclusion criteria. The persons qualified for the study first gave their prior written participation consent and after receiving clear information about the purpose, nature and course of the study.

The study received the Bioethics Committee's approved No. RNN/10/19/KE of January 15, 2019.

The study was conducted in two patient groups. The study group consisted of patients who developed aphasia after stroke. They were referred to the Rehabilitation Clinic of the WAM Teaching Hospital in Łódź for so-called early speech and language (neurological SLA) rehabilitation. The group consisted of 44 patients. They underwent a four-week comprehensive neurological SLA. Six months after the stroke, they received another four-week neurological SLA therapy. The control group consisted of patients who suffered from aphasia after brain stroke. The group consisted of 38 patients. Four-week of neurological SLA therapy started more than six months after the stroke. The patients were ascribed to the groups randomly.

In accordance with the inclusion criterion, the participants of the study were post-stroke patients with cognitive deficits. In the study group, therapy commenced not later than 6 months after stroke ("early therapy"). For the control group, therapy commenced more than 6 months after the stroke ("late therapy").

The exclusion criterion was bi-hemispheric and diffuse brain damage, previous central neurological diseases, psychotic symptoms, severe dementia, as well as lack of patient's informed consent to participate in the study.

The same techniques of neurological SLA therapy were used in both groups of patients.

Language functions were assessed with the Aphasia Dynamics Assessment Scale (Polish abbreviation: SODA).

The SODA scale was developed for diagnostic purposes at the Department of Neurology at Collegium Medicum in Bydgoszcz. Its three parts allow assessing the level of speech comprehension, speech transmission and object naming. Speech comprehension (Part A) is assessed by instructing patients to perform both simple and more complex activities, as well as activities that require understanding of sentence structure. In addition, it is checked whether the respondent correctly interprets well-known proverbs and metaphors. The ability to self-express (part B) is assessed by asking patients to provide personal data, to list automated word sequences, and to repeat simple words, sentences, and numbers. Dialogue and narrative speech also works well. In part C, patients name items that are shown to them. In each of the three parts, participants may obtain a maximum of 3 points. If a patient in part A scored fewer than 3 points, the diagnosis is sensory aphasia. Fewer than 3 points in part B indicates motor aphasia. Fewer than 3 points in part C reveals amnestic (anomic) aphasia. The overall score on the scale is the sum of the scores for speech comprehension, speech production, and naming: SODA X (A + B + C). The sum is indicative of the severity of aphasia. A patient with a score of 0-0.5 is diagnosed with total aphasia. A score of 1-3.5 is evidence of severe aphasia, 4-6.5 of moderate aphasia, and 7-8.5 of mild aphasia. A score of 9 points means no aphasic symptoms were identified [8].

RESULTS

The results of early rehabilitation combined with rehabilitation resumed after six months in the study group was compared with the results of one-time rehabilitation of the control group, whose patients received therapy after at least six months after the stroke. The tables below present mean scores of the test results (± standard deviation) performed in the two groups of patients, before and after therapies. The p values represent test probability obtained by a non-parametric statistical test comparing paired distributions before and after treatment (Wilcoxon's pairwise test). The bottom line shows test probability values in a non-parametric statistical test comparing the distributions in independent samples – the test group and the control group (Mann-Whitney test).

SODA TEST

Each time after a therapy, a statistically significant improvement in the SODA *comprehension* test scores was observed (Table I). This test showed that therapy is effective regardless of the time that elapsed after stroke. In both the control and study groups, patients achieved significant improvement in speech comprehension.

A statistically significant improvement in SODA *naming* scores was observed after each therapy (Table II). The

Table I. SODA aphasia dynamics scale – speech comprehension for patients in the study and control groups in early therapy and after 6 months

SODA comprehension	Early therapy				Therapy after 6 months			
Group	Before	After	Improvement	P	Before	After	Improvement	p
Study	2.3 ± 0.7	2.5 ± 0.6	0.3 ± 0.3	0.0001	2.6 ± 0.5	2.8 ± 0.4	0,2 ± 0,3	0.0007
Control					2.3 ± 0.7	2.6 ± 0.6	0,3 ± 0,4	0.002
P	0.63	0.54	0,94		0.17	0.25	0.21	

Table II. SODA aphasia dynamics scale – object naming for patients from the study and control groups in early therapy and after 6 months.

SODA naming	Early therapy				Therapy after 6 months			
Group	Before	After	Improvement	P	Before	After	Improvement	p
Study	1.5 ± 0.9	1.8 ± 0.9	0.3 ± 0.3	0.0001	1.9 ± 0.7	2.2 ± 0.6	0.3 ± 0.3	00005
Control					1.8 ± 0.9	2.0 ± 0.9	0,2 ± 0,3	0.0003
P	0.89	0.75	0.48		0.039	0.032	0.48	

Table III. SODA aphasia dynamics scale – speech production in patients from the study and control groups in early therapy and after 6 months.

SODA production	Early therapy				Therapy after 6 months			
Group	Before	After	Improvement	P	Before	After	Improvement	P
Study	1.5 ± 0.8	1.8 ± 0.7	0.4 ± 0.4	0.00001	1.9 ± 0.6	2.3 ± 0.6	0.3 ± 0.3	0.00008
Control					1.8 ± 0.7	2.0 ± 0.8	0.1 – 0.3	0.008
P	0.02	0.28	0.004		0.61	0.09	0.002	

Table IV. SODA aphasia dynamics scale – total for patients from the study and control groups in early therapy and after 6 months.

SODA sum	Early therapy				Therapy after 6 months			
Group	Before	After	Improvement	p	Before	After	Improvement	p
Study	5.3 ± 2.0	6.1 ± 1.9	0.9 ± 0.7	0.0000002	6.4 ± 1.7	7.2 ± 1.5	0.8 ± 0.5	0.0000001
Control					5.7 ± 2.0	6.3 ± 1.8	0.6 ± 0.5	0.000003
P	0.5	0.60	0.13		0.11	0.013	0.28	

results proved that the therapy is effective regardless of how much time after stroke rehabilitation is applied. As a result of the therapy, both groups improved their object naming abilities. Importantly, patients in the study group had better results after the repeated (second) neurological SLA rehabilitation compared to the control group.

A statistically significant improvement in SODA *speech production* scores was observed after each therapy (Table III). This test proved the value of speech production therapy for every examined case of aphasia. It is justified to treat every aphasic patient, regardless of the time elapsing since the stroke, because patients in both the control and study group improved their speech production abilities.

Statistically significant improvements in SODA *sum* scores were observed in each therapy (Table IV). The study proved that the condition of patients with aphasia resulting from stroke is improving. Both patients receiving early neurological speech and language therapy and those admitted to therapy six months or more after stroke show progress in regaining their speech comprehension, naming, and speech production. It is important to note, however, that receiving repeated therapy positively and significantly improves the speech production, naming, and comprehension abilities.

DISCUSSION

The aim of this study was to investigate whether combining early, comprehensive and intensive neurological speech and language therapy applied as early as possible in post-stroke patients and continued after six months would result in better recovery of language and cognitive deficits than in the control group, where patients received only late therapy, i.e. later than six months after stroke. It was proven that patients in the study group made significantly and highly significantly greater therapeutic progress than patients receiving only intensive late therapy. Language abilities of patients in both groups before and after therapies were measured by the SODA aphasia dynamics rating scales. SODA comprehension scores showed improvement in both groups, but the total improvement in the study group (after early and late therapy) was 0.5 ± 0.4, which is statistically significantly higher than after a single late therapy in the control group ($p = 0.023$). This was reflected in higher levels of participation in the rehabilitation process due to improved comprehension and greater ability to follow the therapist's instructions. The ability to understand sentence structures also improved. Similar improvements were recorded in the case of the SODA naming scale. As with the previous scale, there were no significant differences in the

parameter distributions of the two groups before the study. However, unlike the SODA comprehension scale, significant differences were observed already after the early (first) treatment of the study group. After the second therapy in the study group and the first (late) therapy in the control group, the total improvement was still significantly higher in the study group. As a result of early and late therapy, the deficits in patients of the study group improved significantly more than those of the control group. Language skills such as giving personal information, repeating simple words, sentences, and numbers, and the ability to join and engage in dialogues enabled better communication and consequently better daily functioning of the patients. Nevertheless, the progress of both groups (not only the study group) in the area of comprehension and naming proves that early intensive therapy combined with late intensive therapy as well as only late therapy are very beneficial in the treatment of aphasia symptoms. In the case of the SODA production scale, the results after the first therapy of the study group did not differ significantly from those of the control group after late therapy ($p = 0.61$), but the difference between the total improvement of the study group and that of the control group was highly statistically significant at $p < 0.000001$. This means that there was evident progress in the patients' ability to spontaneously name objects present in their immediate surroundings. The study showed that intensive neurological SLA therapy is worthwhile regardless of the type of aphasia and the time that has passed between stroke and the onset of symptoms, and that the best results are achieved with a combination of early and late therapy. This is confirmed by the SODA sum scores, as the difference in mean scores after all therapies between the groups was $p = 0.013$, while the total improvement of the study group was statistically highly significantly better than in the control group ($p < 0.000001$).

These results lead to the conclusion that intensive neurological SLA therapy provided to patients as early as possible after stroke and after 6 months results in high improvement of language and cognitive deficits, and that its early provision after stroke gives better results than in the case of therapy introduced later. The findings are consistent with professional literature. It is worthwhile to examine the results of meta-studies in order to be able to draw strong conclusions about the effectiveness of aphasia therapy, since they are based on a large number of patients (although it is important to consider the fact that individual cases of aphasias differ in type, level, details of therapy as well as possible methodological differences between studies of a given meta-study). In a meta-analysis conducted by Brady et al. (2016), researchers extracted data on 57 randomized control trials that included 3002 patients undergoing speech and language therapy. There were 74 randomized comparisons. 27 of them compared patients who underwent speech and language therapy with patients who did not, and it was found that the former group enjoyed clinically and statistically significant benefits in terms of improved functional communication, speech comprehension, and naming. However, these benefits were no longer as evident at follow-up, but this follow-up comparison was based on smaller number of studies). According to 37 randomized comparisons of 1242 patients, intensive therapy, even 15 hours per week or longer, produced significantly better results than low-intensity or shorter therapy, confirming the results obtained in the present study [10].

There is consensus that short time elapsing from stroke to implementation of early intensive post-stroke rehabilitation and aphasia determines the effectiveness of the therapy. It is possible, however, that minimizing the time from stroke occurrence to rehabilitation should not be an end in itself. Coleman et al. cites four animal studies that conclusively demonstrated the harm of undertaking exercise rehabilitation within the first 24 hours after a stroke incident, and that a number of other studies have revealed a beneficial effect of initiating therapy after 24 hours. This would indicate that, at least in animals, the first 24 hours may be a period of high sensitivity of damaged tissues and the nervous system to exercise, and thus immediate rehabilitation might potentially be harmful [11].

The challenge in achieving representative and reliable research results is the multifold nature of aphasia. It takes different forms in different people, so diagnosis, choice of therapy and its objectives are influenced by many factors, e.g. the time elapsed from stroke to commencement of the therapeutic protocol, stroke location, its extent, type and severity of aphasia, type and degree of deficits, applied aphasia classification (there are several), and finally highly individual biological, psychological, linguistic, and social conditions, which differ greatly between patients. Pąchalska states this translates into a methodological problem of compiling a homogenous / reliable patient groups and one from among many possible approaches. As a consequence, a fully clear assessment of the effectiveness of different types of therapy is a challenge. Also, it is often difficult to clearly and unambiguously present positive or negative test results with statistical means. E.g. in this paper's study there were cases where differences in improvement after treatment between the groups measured by a given scale were not statistically significant, although from the clinical point of view the beneficial effects of early and repeated therapy were distinctive in given patients. This may also be indicative of how difficult and long regaining language and cognitive functions is. Often even minimal recovery would prove very important for the patient's functionality and well-being [3].

Two main assumptions of the study were confirmed. Firstly, a combination of early and repeated therapy is more advantageous than only late therapy. This regards improvement of both executive functions necessary to perform complex day-to-day activities and speech functions (understanding, naming and speech production). Secondly, it was demonstrated there is a correlation between patients' biological and social profile and therapy effectiveness. This was also confirmed by UK researchers who proved that speech and language therapy was more effective when it was carried out with high intensity, in a large dose and over a long period of time [10].

Therefore, it is necessary that aphasia treatment is intensive and lasts the longest time possible. In therapeutic efforts, it is crucial to recognize the major role of the family, therapist and, above all, the patients themselves in this quite demanding therapy, which often lasts many years.

CONCLUSIONS

This study investigates the influence of neurological speech and language therapy (neurological SLA) on recovering language and cognitive functions in post-stroke patients. Based on the SODA tests scores, the following conclusions were drawn:

1. In the study group, therapeutic effects were significantly better than in the control group. This was influenced by therapy duration and the fact it was applied twice. It is therefore advisable that aphasia therapy commences as early as possible and lasts as long as possible. This was evidenced by recovery of word memory, re-gaining the ability to understand speech, as well as changes in the degree (severity) of aphasia.
2. For all patients it is highly recommended that they undergo neurological SLA regardless of how much time after stroke has elapsed, as it markedly improves their condition. The therapy led to improved cognitive and language functions in both the study and control group.
3. The sooner the therapeutic measures are implemented and the longer therapy is carried out, the better the results in recovering speech.

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ORIGINAL ARTICLE

PREVALENCE AND RISK FACTORS OF INFERTILITY IN UKRAINE: RESULTS A MULTICENTER STUDY (2019-2021)

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ABSTRACT

The aim: To assess the current prevalence and identify the risk factors for infertility among couples of reproductive age in Ukraine.

Materials and methods: We conducted a retrospective multicentre cohort study was based on reproductive health surveillance data among married populations from January 1st, 2019 to December 31st, 2021 in Ukraine. Definitions of infertility were used from the WHO.

Results: Among all the 6,885 participants in this study, the prevalence of infertility was 25.4%. The prevalence of primary infertility was 5.8%, and the prevalence of secondary infertility was 19.6%. The levels of infertility in the regions of Ukraine had significant differences. It was found that among those women who had primary infertility, more were from rural than urban, while for secondary infertility women the situation was reversed. Infertility was associated with age, history of gynecological surgery, decreased ovarian reserve, age of marriage, long-term air-conditioning environment, and history of endometriosis. There were differences among factors associated with infertility, primary infertility and secondary infertility. The factors associated with primary infertility were age of marriage, age of first sexual intercourse, long-term air-conditioning environment, decreased ovarian reserve and age. A factors associated with secondary infertility were history of gynecological surgery and decreased ovarian reserve.

Conclusions: The results of this study revealed high level the prevalence rate of infertility among couples of reproductive age in Ukraine is high. This applies to both primary and secondary infertility of married women. The most women who had experienced infertility have not sought medical or professional help for the problem.

KEY WORDS: reproductive health, male and female infertility, prevalence, risk factors, family planning, Ukraine

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INTRODUCTION

Infertility is a global socio-economic, demographic, reproductive health and clinical issue affecting millions of people of reproductive age worldwide. Infertility also can cause significant financial loss and emotional stress. Furthermore, infertility is associated with increased risk of subsequent chronic health conditions such as cardiovascular disease [1]. Available data suggests that infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples [2,3], and 186 million individuals [4-6]. However, prevalence estimates of lifetime infertility vary widely, in part because there is no agreed or consistent definition of infertility [7] and because study populations vary by age range, unit of measurement and relationship status [8]. Establishing the true magnitude of infertility as a public health problem is challenging, given that it is not categorized as a disability or chronic condition and may be largely unreported [9].

Fertility is one of the primary components of population dynamics. In the last three decades, the population of Ukraine has been rapidly shrinking due to low birth rates [10]. Within 30 years, its population declined by 10.4 million, from a high of 51.9 million in 1991 to 41.5 million in 2020 [11]. One of the reasons for the low birth rate is the infertility among married population [12]. For this reason decline of fertility level is increasing social interests about infertility as one of priority areas to be addressed not only to respond a potential population aging but also to improve reproductive health in Ukraine. In addition, the declining birth rate could potentially worsen the aging problem in Ukraine.

Infertility is a reproductive healthcare problem which afects not only individuals, families and social populations in Ukraine. Childlessness has still a great social concern which often leads to severe pressure on married couples to have a child. These pressures would more serious each year

of marriage. Despite of significant social meaning of childlessness, there has been less scientific focuses on infertility in sexual and reproductive health field in Ukraine. With a remarkable decline of total fertility rate (TFR), the infertility prevalence rate is considerably high in Ukraine. The introduction of family planning programmers' in Ukraine and other factors derived from socio-economic dynamics did not contributed to a significant increase of the TFR.

Recently, the infertility rate in Ukraine has a trend of increase year by year, and few studies have reported the infertility rate in other regions of Ukraine [13]. However, these data are too old to show the current level in Ukraine, calling for an attempt to measure up-to-date infertility prevalence rate at national level. From our knowledge, only a few studies were conducted to study infertility within narrow clinical features, showing that the percentage of infertile couples seeking infertility treatment services has grown increasingly in Ukraine. Moreover, there has been no attempt to study focused on both of prevalence measurement and investigation of associating risk factors of infertility. In addition, there are did not national data on the prevalence of male and female infertility, as well as primary and secondary infertility among married women in Ukraine.

THE AIM

The aim of this study was to assess the current prevalence and identify the risk factors for infertility among couples of reproductive age in Ukraine.

MATERIALS AND METHODS

DESIGN AND STUDY POPULATION

We conducted a retrospective multicentre cohort study was based on reproductive health surveillance data among married populations from January 1st, 2019 to December 31st, 2021 in Ukraine. We compiled list of the 25 medical centers for family planning and reproductive health. Of these, only 19 medical centers from 17 regions (Lviv, Ivano-Frankivsk, Rivne, Vinnytsia, Volyn, Chernivtsi, Cherkasy, Chernihiv, Poltava, Zhytomyr, Kyiv, Kharkiv, Kherson, Dnipropetrovsk, Donetsk, Luhansk, Odessa) of Ukraine agreed to take part in the study. Cities and villages located respectively in the west and east, and north and south, as well as the in central region of Ukraine were used as objects of comparison, since they often resemble opposing views on social, historical and demographic processes up to the present day. The study population included couples living together and married for more than 1 year, of whom the female spouse was 20-49 years old. Multistage stratified random sampling was used. Participants this study were selected using stratified probability sampling from which couples resident in Ukraine was selected at random. The inclusion criteria for participants were as follows: 20–49 years old; married or cohabitational; local residents. Te exclusion criteria for participants were as follows: never had sexual intercourse; received continuous medical treatment that could afect fertility.

DEFINITIONS

In our study fertility is defined as the ability of a man and woman to reproduce, while infertility denotes lack of fertility or an involuntary reduction in the ability to produce children. Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [14]. In this study infertility among married women was classified as primary and secondary. Primary infertility is the inability to have any pregnancy, while secondary infertility is the inability to have a pregnancy after previously successful conception. In this study 'Thin' means that Body Mass Index (BMI) is less than 18.5 kg/m^2. 'Normal' means 18.5 $kg/m^2 \leq$ BMI < 24 kg/m^2. 'Overweight' means 24 $kg/m^2 \leq$ BMI < 28 kg/m^2. 'Obesity' means that BMI is more than 28 kg/m^2.

DATA COLLECTION

This study includes interviews and questionnaires of couples, also analyses medical records infertile marred women's. We formed a working group to conduce this research, which included obygyne specialists (nurses and physicians) and reproductive medical graduate students. Marred women were approached via telephone and face-to-face in medical centers conversation to complete the standardised and structured questionnaire by trained interviewers. The next step was to complete the paper questionnaires, which contained a series of questions, including basic information, marriage and childbirth history, and personal lifestyle habits, under the guidance of this specialist. During the interviews, participants were introduced this research. The next step was to complete the paper questionnaires, which contained a series of questions and divided into three parts, including basic information, marriage and childbirth history, and personal lifestyle habits, under the guidance of this specialist. Participants had access to free reproductive health counseling in the process. Additionally, anthropometric (height and weight) examinations were performed and recorded by other two specialists. In this study adopted double entry mode of paper questionnaire data and were analysed anonymously. We distributed 8920 paper questionnaires and collected 7113 completed paper questionnaires (79.4%). After excluding those who did not meet the inclusion criteria and who met the exclusion criteria, the final number of study participants was 6885. According to their history of pregnancy, child birth and contraception, the infertile women were divided into primary infertility and secondary infertility cohorts.

ETHICS

This study was performed in line with the principles of the Declaration of Helsinki. The Shupyk National Healthcare

University of Ukraine Ethics Committee approved this study. All participants signed an informed consent and each had a unique identification number assigned to protect their privacy.

STATISTICAL ANALYSIS

In this study the analysis of statistical data was performed using Excel. Prevalence and identify the risk factors for infertility was reported as the percentage of the total number of married women. We conducted descriptive analyses to determine frequencies for categorical variables and calculated medians (or means) for continuous variables. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. We used binary logistic multivariate regression analysis, and for variable selection we used forward stepwise regression based on maximum likelihood estimation. $p < 0.05$ indicated that the difference was statistically significant.

RESULTS

PREVALENCE OF INFERTILITY

During the study period (January 1st, 2019 and December 31st, 2021), of the 6885 participants included in the study, infertility was detected in 1746. The prevalence of infertility in this study cohort was 25.4% (95% confidence interval [CI] 24.9%, 25.9%, p<0.012). The mean ± SD age was 32.9 ± 0.25 years for the fertile group and 34.9 ± 0.39 years for the infertile group. The difference in mean age between these two groups was statistically significant ($p < 0.001$). The levels of infertility in the regions of Ukraine had significant differences. It was detected the high level couples infertility rate in South and Central regions, and low level in West, East and North regions of Ukraine (Table I). The difference in the age, age of marriage, history of gynecological surgery, long-term stay in air-conditioning environment, ovarian dysfunction, and history of endometriosis between the two groups were statistically significant ($p < 0.05$). The infertility rate rose initially and then decreased for every 5 years added to women's age. Women aged 35–39 years had the highest infertility rate. When the participants were grouped by age of marriage, the infertility rate increased as the age of marriage increases. Among all participants the contraceptive prevalence rate was high at all ages, and the rate of women who still hope to conceive decreases as they get older. Characteristics of the difference between the fertile and infertile group are presented in Table II.

The prevalence of primary infertility and secondary infertility was about 5.8% and 19.6%, respectively. Among the infertile women, the difference between primary and secondary infertility was statistically significant by household registry, age, age of marriage, age of first sexual intercourse ($p < 0.05$) (Table III). It was found that among those women who had primary infertility, more were from rural than urban, while for secondary infertility women the situation was reversed. Among infertile women 55.3% went to hospital seeking medical help regardless of the outcome after treatment. From data obtained, the main causes of infertility were fallopian tube factor (27.3%), ovulation disorders (31.7%), endometriosis (18.1%), male factor (13.7%), and unexplained factors (9.2%). Characteristics of primary and secondary infertile groups are shown in Table III.

RISK FACTORS FOR INFERTILITY

In this study Table IV showed the odds ratio (OR) and 95% confidence interval (CI) for the factors associated with infertility in logistic multivariate regression analyses. Infertility was associated with age as shown in logistic regression analysis. Compared with women aged 20–24 years, the adjusted OR for women aged 30–34 years was 5.036 (95% CI, 1.163–21.83) and the adjusted OR for women aged 35–39 years was 6.862 (95% CI, 1.557–30.248). History of gynecological surgery was associated with infertility. Also, ovarian dysfunction was associated with infertility. There were differences among factors associated with infertility, primary infertility and secondary infertility. The factors associated with primary infertility were age of marriage ($p = 0.006$), age of first sexual intercourse ($p = 0.003$), long-term air-conditioning environment ($p < 0.001$), decreased ovarian reserve ($p = 0.005$) and age ($p = 0.002$) A factors associated with secondary infertility were history of gynecological surgery ($p < 0.001$) and decreased ovarian reserve ($p = 0.002$). (data not shown).

Table I. Prevalence of infertility by region of Ukraine (2019-2021)

Region	All (n=6,885)	Fertile (n = 5,139)		Infertile (n = 1,746)		p-value
		n	%	n	%	
West	1,465	1,216	81.7	249	18.3	0.012
East	1,487	1,186	77.4	301	22.6	
North	1,305	1,031	80.7	274	19.3	
South	1,331	912	68.7	419	31.5	
Central	1,297	794	63.7	503	36.3	
Total	6,885	5,139	74.6	1,746	25.4	

Table II. Characteristics of fertile and infertile group in Ukraine (2019-2021)

Characteristics	All (n=6,885)	Fertile (n = 5,139)		Infertile (n = 1,746)		p-value
		n	%	n	%	
Age (years)						
20–24	315	297	5.8	18	1.0	< 0.001
25–29	1,620	1,422	27.7	198	11.3	
30–34	2,358	1,629	31.7	729	41.8	
35–39	1,341	855	16.6	486	27.8	
40–44	855	639	12.4	216	12.4	
45–49	396	297	5.8	99	5.7	
Place of residence						
Urban	3,609	2,715	52.8	894	51.2	0.569
Rural	3,276	2,424	47.2	852	48.8	
Occupation						
Unemployed	936	666	13.0	270	15.5	0.541
Head of enterprises	972	711	13.8	261	14.9	
Professional worker	2,574	1,980	38.5	594	34.0	
Clerk	261	216	4.2	45	2.6	
Service worker	945	711	13.8	234	13.4	
Agricultural worker	180	117	2.3	63	3.6	
Operator	45	36	0.7	9	0.5	
Other	972	702	13.7	270	15.5	
Educational level						
Primary	972	675	13.1	297	17.0	0.391
High school	1,044	819	15.9	225	12.9	
Junior college degree	1,512	1,150	22.4	362	20.7	
Bachelor's degree and above	3,357	2,495	48.6	862	49.4	
Smoking						
No	90	72	1.4	18	1.0	0.582
No, secondhand smoke	1,602	1,251	24.3	351	20.1	
Yes	5,193	3,870	75.3	1,323	75.8	
Drinking						
No	801	666	13.0	135	7.7	0.072
Yes	6,084	4,473	87.0	1,611	92.3	
BMI[a] (kg/m^2)						
Thin	495	351	6.8	144	8.2	0.486
Normal	4,347	3,303	84.3	1,044	59.8	
Overweight	1,557	1,125	21.9	432	24.7	
Obese	486	360	7.0	126	7.2	
Age of marriage (years)						
≤ 24	3,429	2,717	52.9	712	40.8	0.041
25–29	3,177	2,251	43.8	926	53.0	
≥ 30	279	171	3.3	108	6.2	
Age of first sexual intercourse						
< 20	864	702	13.7	162	9.3	0.197
20–25	4,545	3,384	65.8	1,161	66.5	
> 25	1,476	1,053	20.5	423	24.2	
History of gynecological surgery						
No	6,183	4,788	93.2	1,395	79.9	< 0.001
Yes	702	351	6.8	351	20.1	
History of endometriosis						
No	6,696	5,031	97.9	1,665	95.4	0.048
Yes	189	108	2.1	81	4.6	
Long-term air-conditioning environment						
No	2,601	1,827	36.6	774	44.3	0.009
Yes	4,284	3,312	64.4	972	55.7	
DOR[b]						
No	6,525	4,995	97.2	1,530	87.6	< 0.001
Yes	360	144	2.8	216	12.4	
Total	6,885	5,139	74.6	1,746	25.4	

[a]BMI, Body Mass Index

[b]DOR, Decreased ovarian reserve

Table III. Characteristics of primary and secondary infertile groups in Ukraine (2019-2021)

Characteristics	All (n=1,746)	Primary infertility (n = 472)		Secondary infertility (n = 1,274)		p-value
		n	%	n	%	
Age (years)						
20–24	18	18	3.6	0	0.0	< 0.001
25–29	198	143	30.3	55	4.3	
30–34	729	189	40.0	540	42.4	
35–39	486	91	19.3	395	31.0	
40–44	216	22	4.7	194	15.2	
45–49	99	9	1.9	90	7.1	
Place of residence						
Urban	868	173	36.7	695	54.6	0.014
Rural	878	299	63.3	579	45.4	
Age of marriage (years)						
≤ 24	702	135	28.6	567	44.5	0.014
25–29	936	274	58.1	662	52.0	
≥ 30	108	63	13.3	45	3.5	
Age of first sexual intercourse (years)						
< 20	182	36	7.6	146	11.5	< 0.001
20–25	1172	207	43.9	965	75.7	
> 25	392	229	48.5	163	12.8	

Table IV. Logistic multivariate regression analyses of risk factors associated with infertility in Ukraine (2019-2021)

Characteristics	p-value	Unadjusted OR[a] (95% CI)	p-value	Adjusted OR (95% CI)
Age (years)				
	< 0.001		< 0.001	
20–24		Ref		Ref
25–29	0.269	2.297(0.515–10.249)	0.587	1.523 (0.335–6.943)
30–34	0.012	6.618(1.549–28.274)	0.031	5.036 (1.163–21.83)
35–39	0.003	9.379 (2.165–40.619)	0.011	6.862 (1.557–30.247)
40–44	0.025	5.577 (1.244–25.011)	0.109	3.49 (0.758–16.071)
45–49	0.035	5.50 (1.131–26.752)	0.174	3.096 (0.607–15.797)
History of gynecological surgery				
No		Ref		Ref
Yes	< 0.001	3.611 (2.234–5.831)	< 0.001	3.063 (1.819–5.158)
DOR[b]				
No		Ref		Ref
Yes	< 0.001	5.131 (2.662–9.878)	< 0.001	3.835 (1.908–7.712)
Constant			0.003	0.109

[a]OR, Odd Ratio
[b]DOR, Decreased ovarian reserve

DISCUSSION

This is the first multicentre study was aimed to estimate the prevalence and both primary and secondary infertility rate among Ukrainian married women and identify the factors associated with vulnerability of infertility. In this study the current prevalence of infertility among couples of reproductive age in Ukraine was 25.4%. The prevalence of primary infertility among women was 5.8%, and the

prevalence of secondary infertility was 19.6%. The levels of infertility in the regions of Ukraine had significant differences. It was detected the high level couples infertility rate in South and Central regions, and low level in West, East and North regions of Ukraine. It was found that among those women who had primary infertility, more were from rural than urban, while for secondary infertility women the situation was reversed. Infertility was associated with age, history of gynecological surgery, ovarian dysfunction, age of marriage, long-term air-conditioning environment, and history of endometriosis. There were differences among factors associated with infertility, primary infertility and secondary infertility. The factors associated with primary infertility were age of marriage, age of first sexual intercourse, long-term air-conditioning environment, decreased ovarian reserve and age. A factors associated with secondary infertility were history of gynecological surgery and decreased ovarian reserve.

Reports about the prevalence of infertility are rare currently in Ukraine. According to national statistical reports, the prevalence of current infertility has increased in 3.0 times for the last five years (2012-2017) in Ukraine. Our estimates of infertility are broadly in line with those found by previous studies although prevalence estimates differ as a result of diverse definitions and study populations.

The prevalence of infertility varies greatly in different countries and regions, and change all the times. The infertility estimates use different definitions considering different periods, which make direct comparisons difficult between various studies. The definition, as well as the etiological causes associated with infertility, differs from region to region. Infertility affects both men and women, and approximately 10% to 15% of couples in industrialized countries are infertile. The percentage of infertile men ranged from 2-5% to 12% [7]. Infertility rates were highest in Africa and Central/Eastern Europe. Additionally, according to a variety of sources, rates of male infertility in North America, Australia, and Central and Eastern Europe varied from 4 5-6%, 9%, and 8-12%, respectively [7]. The infertility prevalence rate was in the United States 15.5% [15], in Canada 11.5-15.7% [16], in Britain 12.5% [8], in China 24,6% [17]. In Scotland, nearly one in five women attempting conception experienced infertility [18]. The percentage of infertility, based on data collected in demographic surveys, varies between different countries as well as gender, with the higher being in men, examples being >10% and 17% for Finland and 18% and >21% for Switzerland [13]. Prevalence of infertility among women in Norway, Poland, Romania, Czech Republic has been recorded as between 5–8%, which increases to >10% in Sweden and Canada. The large differences among countries could be attributable to large differences involuntary infertility [13]. Other studies have reported higher and lower estimates but, because of differences in study groups and outcome measures, comparison is not possible. In our study prevalence of infertility was 25,4%.

Previous studies have shown that age, history of gynecological surgery, decreased ovarian reserve, age of marriage, long-term air-conditioning environment, and history of endometriosis, age of first sexual intercourse are factors associated with infertility [17]. Fertility problems were associated with endometriosis and pelvic surgery [17, 18]. The risk of primary infertility proportionally increases by age since reproduction capability is biologically decrease when a woman ages [19]. Some former studies also demonstrated that young women were frequently exposed to sexual dysfunction and ovulatory disorders, which had already been known as leading causes of infertility [17, 20]. The risk factors of infertility detected in our study are comparable with those obtained in other previous similar studies.

We found that ever experience of infertility and of help seeking were associated with few current health factors for women. In Ukraine little more than half of women who had experienced infertility had sought medical or professional help for the problem. Those who did so were better educated and in higher status occupations. Our estimate that 55.3% of women sought medical help for infertility is close to that reported by an international review which estimated that 56% of women in more developed countries sought help [5], and by a Finnish study [21], and by a Britain study [8] which found that 57% of all subfertile women did so. Other studies [22, 23] have reported higher and lower estimates but, because of differences in study groups and outcome measures, comparison is not possible.

According to literature, interventions to encourage help seeking include raising public awareness about reproductive risks and strategies to minimize them [24], general practitioners taking opportunities to discuss fertility with patients, greater access to fertility treatments [25]. Ukraine is a country that lays particular emphasis on the issue of infertility, especially after experiencing a dramatic fertility decline over the last two decades. The Ukrainian State program for infertility treatment by budget funds was approved in Ukraine since 2004. However, most infertile women do not have access to this program. This means that many women have to pay out-of-pockets. Reproductive healthcare for young women should be strengthened with promotion of health education to provide them with sufficient knowledge related to reproductive health problems including potential risk factors to develop primary infertility.

STUDY STRENGTHS AND LIMITATIONS

Our study investigation has several strengths. First, this is the first multicentre study was aimed to estimate the prevalence and both primary and secondary infertility rate among Ukrainian married women and identify the factors associated with vulnerability of infertility. Second, size of the sample and the fact that it is population-based. Our findings will be useful to resource allocation and health services planning for the growing number of patients with infertility. However, the limitations of this study also need to be noted. First, the estimation of prevalence of infertility was based on questionnaire-based interview method. The

current study relied on women's response to these questionnaires; these assumptions may be inaccurate, as women may not reveal accurately on this sensitive topic such as past voluntary abortions. Self-reported data may be subject to recall bias. Second, men were not included in the study, although they may also cause infertility. Despite some limitations, this study is helpful to understand infertility dynamics and some influencing factors increasing the risk of infertility in Ukraine. We expect that our findings will be invaluable to health professionals toward their efforts to reduce the burden of infertility in their respective regions of Ukraine.

CONCLUSIONS

The results of this study revealed high level the prevalence rate of infertility among couples of reproductive age in Ukraine is high. This applies to both primary and secondary infertility of married women. The most women who had experienced infertility have not sought medical or professional help for the problem. The main reason for this is the high cost for infertility treatment. There should be taken practical measures at policy level for education to improve literacy level related to sexual and reproductive health including prevention of infertility among young women. There should be developed a specific health policy to focus on the support for infertile couples to promote better access to infertility diagnosis and treatment service in Ukraine. Further research is required to determine which set of interventions minimize factors increasing the risk of infertility.

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ORIGINAL ARTICLE

IMPACT OF FGFR4 (GLY388ARG) GENE POLYMORPHISM ALONG WITH VISFATIN CYTOKINE AND HIGH MOBILITY GROUP BOX-1 (HMGB1) ON ACUTE CHOLECYSTITIS

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ABSTRACT

The aim: Evaluating the role SNP rs351855 of (FGFR4) gene and estimating the serum concentration of Visfatin cytokine and (HMGB-1) protein in AC patients and in healthy control blood samples.

Materials and methods: Blood samples were collected from 35 patients and 35 healthy controls, and then the serum was used for ELISA test, another each 2 ml blood were used for DNA extraction and rs351855 of (FGFR4) PCR assay.

Results: there was no significant difference in mean HMG and mean visfatin among (FGFR4) rs351855 genotypes in patients and control group. There was no significant difference in mean (HMG) among (FGFR) rs351855 genotypes in patients` group ($p = 0.923$); there was also no significant difference in mean visfatin among FGFR rs351855 genotypes in patients` group (p=0.161) rs351855 genotypes showed that the homozygous GG, heterozygous A/G and homozygous AA. Despite these minor differences there was no significant variation ($p = 0.323$), also no significant difference in frequency distribution of individuals according to FGFR rs351855 G>A SNP polymorphism between patients` and control groups ($p = 0.454$). The same was applied to recessive and allelic analysis p>0.05.

Conclusions: There was no role for (FGFR4) rs351855G/A SNP in disease susceptibility to acute cholecystitis in Iraqi patients. Visfatin cytokine and HMGB-1 protein might act as a good biomarker for diseases.

KEY WORDS: Acute cholecysitis, Fibroblast growth factor, receptor 4(rs351855) gene polymorphism, Human HMGB-1, Human VF

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INTRODUCTION

Acute cholecystitis an inflammation of gallbladder [1] resulted from obstruction of the gallbladder neck or cystic duct by a gallstone or by parasitic worms, mucus or endoscopic stenting of bile ducts [2-3]. The most common factor is interaction between genetic and environmental factors [4] along with stimulation of adaptive immunity by Th1- mediated proinflammatory immune response and secretion of cytokines. In addition to that innate immunity also had an important role in defense mechanism against cholecystitis represented by Toll-Like receptors. AC is the most popular surgical diagnostic in developing countries; it is present as AC in up to 20% [5]. More than 90% of cases of AC are associated with cholelithiasis; in the remaining 10% of cases, cholecystitis occurs in the absence of gallstones, which is termed AC [1]. White Americans, Asians, African Americans and Africans are less likely to have AC [6]. The cause of AC involves immune stimulation of epithelial gallbladder cells; T-cell seems to play central role. Family history, estrogen treatment, pregnancy and diabetes are common, as well as certain genes and human leukocyte antigens may have significant role. Especially HLA DRB1 plays a key role in the immune system in AC [7]. Visfatin is one of the soluble mediators that are produced by adipocytes and manifest their roles in an autocrine, paracrine or endocrine manner [8]. Visfatin can be considered early prognosis factor for AC, as expression increased in the shortest period of time relative to other pro-inflammatory mediators, including IL-10, TNF-α, IL-6, ICAM-1 and VCAM [9]. The role of HMGB1 as an important biomarker in enhancing the diagnostic accuracy of AC when used in combination with normal diagnostic tests was highlighted in [10].

THE AIM

Thus, the aim of present study is to find out effect of the bile acid regulator FGFR4 SNP, on disease susceptibility along with its effect on serum concentration of visfatin cytokine and high mobility group box protein 1 (HMGB1) in acute of cholecystitis patients.

MATERIALS AND METHODS

PATIENTS AND SAMPLE COLLECTION

A case control study was conducted in AL-Diwaniyah province, in 35 patients with AC, who included 8 males and 27 females, who attended the consultant general surgery in AL-Diwaniya teaching hospital with further reviewing patients at AL -Furat

Table I. The ARMS-PCR primers with their sequence and amplicon size

Primer	Sequence (5'-3')	Product size
Wild type A allele Forward Primer	CTGCCCTCGATACAGCA	260bp
Mutant G allele Forward Primer	CTGCCCTCGATACAGCAC	
Common Reverse Primer	CTGGGCATAACTACAGCTTC	

Table II. The mean age and age range and frequency distribution of patients and control group according to gender

Characteristic	Control n = 35	Patients n = 35	p
Age (years)			
Mean ±SD	42.83 ±11.90	42.29 ±12.12	0.851 I NS
Range	25 -67	21 -68	
Gender			
Male, n (%)	8 (22.9 %)	8 (22.9 %)	1.000 C NS
Female, n (%)	27 (77.1 %)	27 (77.1 %)	

n: number of cases; SD: standard deviation; I: independent samples t-test; C: Chi-square test; NS: not significant at p> 0.05

Table III. Frequency distribution of patients and control subjects according to smoking

Smoking	Control n = 35	Patients n = 35	p
Yes, n (%)	7 (20.0 %)	30 (85.7 %)	<0.01 HS
No, n (%)	28 (80.0 %)	5 (14.3 %)	

n: number of cases; SD: standard deviation; C: Chi-square test; HS: highly significant at p> 0.05

Table IV. Comparison of mean high mobility group (HMG) and mean visfatin between patients with acute cholecysitis and control group

Characteristic	Control n = 35	Patients n = 35	p
HMG (pg/ml)			
Mean ±SD	1768.10 ±366.72	2246.10 ±89.75	< 0.001 I HS
Range	766.11 -2481.15	1978.22 -2292.99	
Visfatin (pg/ml)			
Mean ±SD	10.64 ±6.00	16.33 ±3.55	< 0.001 I HS
Range	1.46 -24.22	12.19 -23.97	

n: number of cases; SD: standard deviation; I: independent samples t-test; HS: highly significant at p ≤ 0.01

Table V. Frequency distribution of patient with acute cholecysitis and control subjects according to FGFR rs351855 G>A SNP polymorphism

Mode	FGFR rs351855 G>A	Control n = 35	Patients n = 35	p	OR	95% CI
Co-dominance	GG	24(68.6%)	21(60.0%)	0.323 C NS	Reference	
	A/G	9(25.7%)	8(22.9%)		1.02	0.33-3.11
	AA	2(5.7%)	6(17.1%)		3.43	0.62-18.85
Dominant	GG	24(68.6%)	21(60.0%)	0.454 C NS	0.69	0.26-1.84
	A/G+AA	11(31.4%)	14(40.0%)		Reference	
Recessive	GG+A/G	33(94.3%)	29(82.9%)	0.133 C NS	Reference	
	AA	2(5.7%)	6(17.1%)		1.45	0.57-3.69
Alleles	G	57(81.4%)	50(71.4%)	0.163 C NS	Reference	
	A	13(18.6%)	20(28.6%)		1.14	0.50-2.58

n: number of cases; C: Chi-square test; NS: not significant at p> 0.05; OR: odds ratio; CI: confidence interval

National Hospital in the period from 10 December 2020 to 15 February 2021 under the supervision of general surgery doctors. Blood samples were collected by venipuncture from 35 patients and 35 healthy controls, five milliliters of venous blood were drawn by disposable syringe under aseptic technique. Three ml of blood were put in gel tube and allowed to clot; then the serum was separated by centrifugation 1500 rpm for 5 minutes. The serum has been collected in Eppendorf tube without

Table VI. The level of HMGB-1 and visfatin according to FGFR4 rs351855 G>A SNP polymorphism

Group	Characteristic	AA n = 2	A/G n = 9	GG n = 24	p
Control	HMG (pg/ml)	1766.40 ±359.18	1826.50 ±231.16	1746.40 ±419.29	0.923 K NS
	Visfatin (pg/ml)	14.05 -2.06	13.45 -7.87	9.30 -5.06	0.161 K NS
Group	Characteristic	AA n = 6	A/G n = 8	GG n = 21	p
Patients	HMG (pg/ml)	2274.10 ±2.23	2254.30 ±68.26	2240.70 ±100.81	0.863 K NS
	Visfatin (pg/ml)	16.77 -3.61	17.60 -4.18	15.73 -3.32	0.341 K NS

n: number of cases; data were presented as mean standard deviation; K: Kruskal Wallis test; NS: not significant at p> 0.05

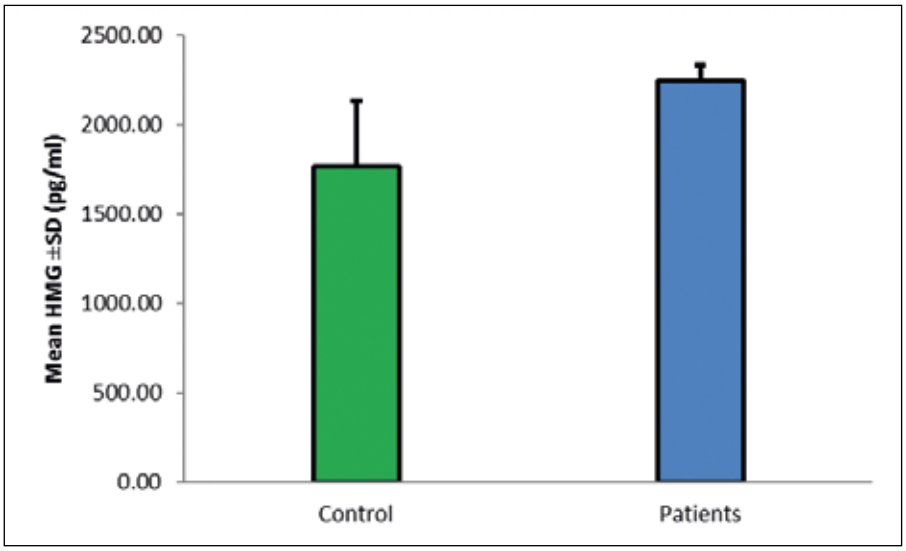

Fig 1. Comparison of mean high mobility group (HMG) between patients with acute cholecystitis and control group

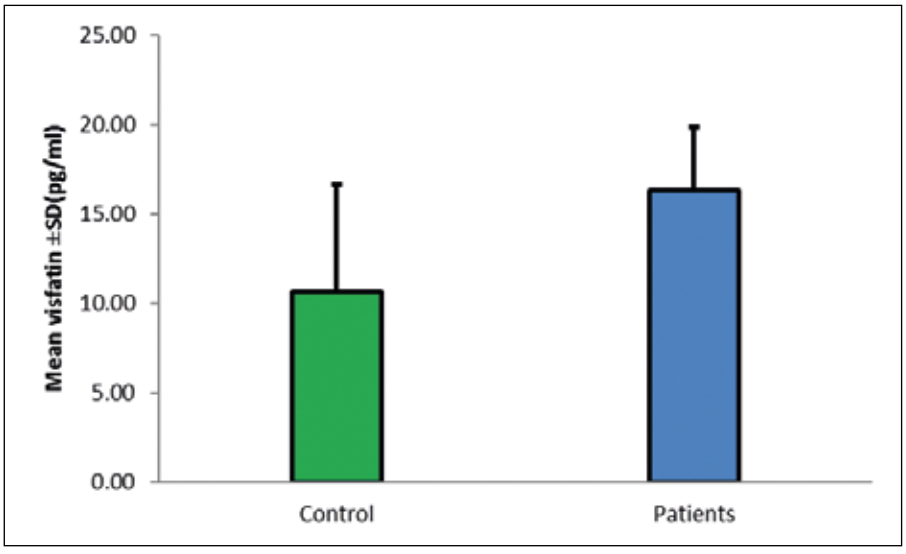

Fig 2. Comparison of mean visfatin between patients with acute cholecystitis and control group

ethylinditetracitic acid EDTA, then stored at -20°C to be used for ELISA test to determine concentration of Visfatin cytokine and HMGB-1 protein in serum of patients. Another two ml of each blood sample were collected in EDTA tube and stored at -4°C for DNA extraction by using (gSYAN DNA kit extraction kit Geneaid Taiwan) for detection of FGFR4Gly388Arg polymorphism by Allele-specific PCR study design.

TOTAL DNA EXTRACTION

Genomic DNA from blood samples were extracted by using (gSYAN DNA kit extraction kit Geneaid Taiwan) (Frozen Blood) Geneaid USA, and procedure was done according to company instructions. The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO USA), which measured DNA

concentration ng/µL and check the DNA purity by reading the absorbance at 260/280 nm.

ARMS PCR TECHNIQUE

Amplification-refractory mutation system-polymerase chain reaction technique ARMS-PCR assay was performed for detection and genotyping of FGFR (rs351855) gene polymorphism in acute cholecystitis patients and in healthy control blood samples, this method was carried out according to one described by [11].

PRIMERS

Amplification - refractory mutation system-polymerase chain reaction technique ARMS-PCR primers FGFR (rs351855) gene polymorphism was designed in this study using NCBI-SNP data base and Primer1 ARMS-PCR primers design online. These primers were provided from (Scientific Researcher. Co. Ltd. Iraq) as given in the table I.

ELISA OF VISFATIN CYTOKINE AND HMGB-1 PROTEIN

Following the manufacturer's instructions, the concentration of HMGB1 in the serum was determined using a commercially available ELISA kit (Elabscience®-test, USA).

RESULTS

THE MEAN AGE AND AGE RANGE AND FREQUENCY DISTRIBUTION OF PATIENTS AND CONTROL GROUP ACCORDING TO GENDER

There was no significant difference in mean age between patients and control group (p = 0.851). As well as the control group was selected in such a way to include comparable number of males and females to patients' group, thus there was no significant difference in frequency distribution of patients and control group according to gender (p = 1.000), table II.

FREQUENCY DISTRIBUTION OF SMOKERS IN PATIENTS AND CONTROL SUBJECTS

The patient group included 5(14.3%) smokers and 30 (85.7%) non-smokers, whereas the control group included 7 (20.0%) smokers and 28 (80.0%) non-smokers. There was found a significant difference in the frequency distribution of patients and control subjects according to smoking p<0.01, as shown in table III.

COMPARISON OF MEAN HIGH MOBILITY GROUP BOX-1PROTEIN (HMGB-1) AND MEAN VISFATIN CYTOKINE (VS) BETWEEN PATIENTS WITH ACUTE CHOLECYSTITIS AND CONTROL GROUP

The results are shown in table IV. The mean HMG of patients group was higher than that in control group (2246.10±89.75 pg/ml versus 1768.10±366.72 pg/ml), respectively, in a highly significant manner (p<0.001), as shown in figure (1). The mean visfatin of patients group was higher than that of control group, 16.33±3.55 pg/ml versus 10.64±6.00 pg/ml, respectively, in a highly significant manner (p<0.001), as shown in figure (2).

GENETIC STUDY

FREQUENCY DISTRIBUTION OF PATIENT WITH ACUTE CHOLECYSTITIS AND CONTROL SUBJECTS ACCORDING TO FGFR RS351855 G>A SNP POLYMORPHISM

As shown in table V, regarding the FGFR4 rs351855 genotypes in Iraqi acute cholecystitis patients showed the homozygous GG, heterozygous A/G and homozygous AA. Despite these minor differences there was no significant variation, p=0.323. Also, no significant difference in the frequency distribution of individuals according to FGFR rs351855 G>A SNP polymorphism between patients` and control groups was found (p=0.454); the same was applied to recessive and allelic analysis - p>0.05.

THE LEVEL OF HMG AND VISFATIN ACCORDING TO FGFR RS351855 G>A SNP POLYMORPHISM

As shown in table VI, there was no significant difference in mean HMG and mean visfatin among FGFR4 rs351855 genotypes in patients` and control group. There was no significant difference in mean HMG among FGFR rs351855 genotypes in patients` group (p = 0.923), there was also no significant difference in mean visfatin among FGFR rs351855 genotypes in patients` group (p = 0.161).

DISCUSSION

The current study found that AC was not limited to a specific age group, as the study group's ages ranged from 21 to 68 years, suggesting that AC could occur in both youth and elderly communities [12-13]. This agrees with Tokyo recommendations that do not regard advanced age as a risk factor per se, although they underline the likelihood of older patients to experience serious AC [14]. Our study indicated that female gender is at risk factor of AC and that AC occurred in women more than in men. That corresponds with many studies [15-16]. This occurs due to several reasons hormone, pregnancy, obesity in pregnancy raise the likelihood that females can develop cholelithiasis in future [17].

Estrogen has been shown to result a two-fold increasing of gallstone formation, compared to males, due to increasing of bile cholesterol as well as a decreasing of gallbladder contractility in women of reproductive age or those under estrogen-containing birth control medication [18]. Women have more robust immune responses to both foreign and self-antigens [19]. Smoking may increase gallbladder disease risk by reducing plasma high density lipoprotein cho-

lesterol concentrations as higher levels of HDL cholesterol are associated with lower gallbladder disease risk [20]. The (HMGB1) has been known to hold critical roles in pro-inflammatory reactions [21]. HMGB1 is pro-inflammatory as secreted by innate immune cells, including macrophages in the early stages of sepsis. It can also promote cell survival by inducing defensive autophagy [22].

The present result is similar to two previous studies conducted by [10]. It was found the serum concentration of HMGB-1 was significantly higher in AC patients and also mentioned that the HMGB1 levels may act as an effective biomarker for prediction of AC complementary to other diagnostic tests [10]. In acute cholecystitis, it is conceivable that the release of humoral mediators from the excessive activated macrophages/monocytes may lead to remote organ injury, as the released HMGB-1 can cause the development of inflammation [11].

Concerning visfatin is a pro inflammatory cytokine (key-cytokine) released from the peripheral blood mononuclear cells (PBMCs) as well as adipose tissue [9]. AC patients exhibited significant higher levels of visfatin in serum group than in normal group. This agrees with the study done by [9]. However, visfatin can inhibit the expression of IL-6, IL-1β, IL-10 and IL-4 and promote TNF-α in presence of LPS [23], suggested that visfatin could be responsible for the overproduction of TNF-α in patient with low-grade inflammation.

This is because visfatin forms cell-cell contacts with T cells, APCs such as monocytes, DCs, and macrophages (Riquelme et al., 2018). Visfatin has been shown to suppress the expression of ABCG1 in gallbladder epithelial cells (GBECs), which increases the inflammatory response during AC [9]. High plasma levels of visfatin are associated with systemic inflammation during AC (Xie et al., 2014), stimulating the expression of interleukin-6 in amniotic cells [24] via the p38 mitogen-activated protein kinase and p38 Mitogen-activated protein kinase 1 pathways [25]. According to the genetic study we found no significant association between FGFR4 (rs351855) gene polymorphism and susceptibility to AC in Iraqi patients. This correlates with results of previous study done by [26] among gallstone patients in Iraqi population. And also agrees with study done by Chen et al., 2012 among gallstone patients in Chinese population [27]. Findings suggest that FGFR4 genotype variant of rs351855 would be not contributed to disease susceptibility of acute cholecystitis in Iraqi patients.

A new study has identified a genetic risk factor that contributes to the aggravation of gallstone disease, called FGFR4Gly388Arg (G-388R). It has increased inhibitory activity and polymorphism in the gene expression of cholesterol 7α-hydroxylase (CYP7A1) (Abdeldayem, 2017), and then increased phosphorylation after ligand binding [11].

The cystic duct is an area of the gallbladder where cholesterol supersaturated bile leads to the obstruction. This goes to intraluminal pressure within the gall bladder, triggering an intense inflammatory response in the body's immune system [28], manifested by cell-mediated immunity (Th1 cell) and secretion of cytokines and increased development of immunoglobulin's that modify mucin production [29], which has an effect on the development of gallstone cholesterol and local inflammation. It was first proven by [30]. The types of mediators and immune cells involved are all variable and depend on several factors such as the type of promoter, duration of injury, and multiple genetic sites [31]. The FGFR4 gene polymorphism does not have a significant role in occurrence of acute local inflammation in the gallbladder, according to the control group in Iraqi population. However, the presence of local inflammation is associated with an immune response and stimulation of immune cells, which leads to releasing of lots of cytokines into inflammatory area, including HMGB1 and Visfatin cytokines. However, there was no significant difference between FGFR rs351855 genotypes in the patient group in mean HMG ($p = 0.923$) and Visfatin ($p = 0.161$).

CONCLUSIONS

The present results concluded that there was no role for (FGFR4) rs351855G/A SNP in disease susceptibility to acute cholecystitis in Iraqi patients. Visfatin cytokine and (HMGB-1) protein play a critical role of the pathogenesis of acute cholecystitis. Thus, they could act as a good biomarker for diseases.

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ORIGINAL ARTICLE

INTERDEPENDENCE OF THE MICROBIOCENOSE COMPOSITION OF BIOPELLICLE AND THE SEVERITY DEGREE OF CHANGES IN THE MUCOSA OF THE GUMS AFTER TEN WEEKS OF EXPERIMENTAL OPIOID EXPOSURE

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ABSTRACT

The aim: To study the relationship between the degree severity of changes in the tunica mucosa of the rats gums and the composition of the microbiota of tooth surface in the gingival margin under the ten-week action of the opioid.

Materials and methods: The study was performed on 34 male rats, weighing 160 - 255 g, aged 4.5 - 7 months. Animals were administered nalbuphine for 10 weeks, gradually increasing the dose (0.212 - 0.283 mg/kg). The research of the bacterial from the microbiotope of tooth surface in the gingival margin of rats was performed using statistical analysis of quantitative results.

Results: The 4 stages of development of the pathological process and changes in the qualitative and quantitative composition of the microbiocenosis in the gingival margin of the oral mucosa are determined. Depending on the severity of pathological changes in the mucous membrane of the gums of rats under the long-term action of the opioid, a significant increase in the quantitative indicators of bacterial species of pathogenic and opportunistic microbiota at degree IV of the process compared with degree I. The appearance of potential pathogens of purulent-inflammatory processes - *Klebsiella* and *Pseudomonas aeruginosa* was noted.

Conclusions: The relationship between the quantitative and qualitative characteristics of the microbiocenosis of tooth surface in the gingival margin and the severity of changes in the mucous membrane of the of rats gums under the ten-week action of the opioid was determined, indicating bacterial associations of dental biopellicle as an etiological factor.

KEY WORDS: microbiota, opioid, rats, mucosa, gums, degree of severity of the process

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INTRODUCTION

Over the last decade, there has been an increase in illegal drug use and uncontrolled opioid abuse in patients with chronic pain, which is associated with unintentional trauma and is a major risk factor for tolerance and withdrawal, leading to overdose and death [1-5]. The attention of scientists in various fields of medicine is focused on the study of changes in organs and systems under the influence of drugs, in particular, both in clinical and experimental areas [6-9]. In clinical studies it is indicated that when exposed to opioids there are signs of immunosuppression, which cause an increased risk of infectious diseases and the development of inflammation [5, 10, 11]. Toxic effects of drugs are manifested in all organs and systems, which may have an indirect or direct effect on the organs of the oral cavity [6, 12-15].

The question of the role of bacterial flora in the etiology and initiation of periodontal disease is certainly actively studied as the improvement of microbiological methods and the accumulation of research results [16-19]. Today, one of the main hypotheses remains that dental plaque microorganisms are a determining factor in the development and progression of the inflammatory process in the periodontium, which provoke the inflammatory process and directly affect the microbial status of the oral cavity [20-23].There is also evidence that the role of microorganisms in the development of periodontitis is unclear, although some bacterial pathogens alone or as part of microbial groups may be particularly important [24].

Therefore, in order to prevent the development of periodontal disease and the occurrence of infectious foci in the oral cavity caused by bacterial biopellicle, it is important to determine the etiology and pathogenesis of this pathology in experimental animal models in order to further extrapolate these data to the clinic [25]. However, the relationship between the species and quantitative composition of the

microbiota of tooth surface in the gingival margin and the development and progression of inflammation in the gingival mucosa under action of the opioid are controversial and needs further study using modern methods of microbiological research in the experiment.

THE AIM

To investigate the relationship between the severity degree of pathomorphological changes in the mucous membrane of the gums and the composition of the microbiocenosis of the tooth surface in the gingival margin after ten weeks of action of the opioid in the experiment.

MATERIALS AND METHODS

The study was performed on 28 white mature nonlinear male rats, weighing 160 - 255 g, aged 4.5 - 7.0 months. In the experiment, the animals were divided into 2 main groups. Group I: intact rats (10). Group II consisted of 18 animals, which was administered an opioid analgesic for ten weeks. Animals were administered the opioid analgesic Nalbuphine with the active substance nalbuphine hydrochloride (dilution of 1 ml of nalbuphine hydrochloride in 50 ml of 0.9% sodium chloride solution) intramuscularly, daily, once, at one time interval for 10 weeks, with a gradual increase in dose every 2 weeks: for 1-2 weeks - 0,212 mg / kg, 3-4 weeks - 0,225 mg / kg, 5-6 weeks - 0,252 mg / kg, 7-8 weeks - 0,260 mg / kg, 9-10 weeks - 0,283 mg / kg.

Microbiological studies included bacterioscopic and bacteriological methods to study the features of the microbiocenosis of the oral cavity of intact rats and rats under the influence of opioid analgesics. To conduct microbiological studies, smears were selected to study the bacterial from the mikrobiotope of the tooth surface in the area of the gingival margin, namely at the site of biopellicle formation. In bacterioscopic studies, smear material was applied to a glass slide, fixed over a burner flame, and Gram stained. In order to obtain the level of microbial colonization was carried out by sampling with a calibrated loop of primary material (0.02 ml) and transferred to a test tube with isotonic solution (1 ml). This procedure was repeated 5 times from this mikrobiotope. In 0.2 ml of saline contained the number of microorganisms that were introduced in one calibrated loop - 0.02 ml. In the laboratory, 0.2 ml of material was taken from a test tube and inoculated into 5 Petri dishes with a dense nutrient medium. Accordingly, when counting colonies, we obtained a quantitative indicator in colony-forming units (1 colony - 1 CFU). Conventional special, differential diagnostic and selective media were used: meat-peptone agar, blood agar, Endo medium, Saburo medium, yolk-salt agar, mannitol salt agar. After 24 - 48 hours the number of colonies was counted. Cultivation was performed under normal atmospheric conditions, as well as at elevated CO_2 levels. After counting, the characteristic isolated colonies were subcultured on inclined agar to obtain pure cultures. To create the necessary gas composition for anaerobes, the cups were placed in a microanaerostat from Bio Merieux with packages and cultured for 48 hours. at t 37 ° C, after that the number of colonies was counted.

The obtained data, for further statistical analysis, were tested for normality by calculating the coefficients of asymmetry and excess, using the Shapiro-Wilk test ($p < 0.05$). The central trend for all data was presented as M ± SD (mean ± standard deviation). To determine the significance of the difference between three or more groups of animals, ANOVA analysis of variance with the post-Hawk Tukey test was used for further pairwise comparison. If the distribution of data, according to the results of the test, differed from normal, then to establish the reliability of the difference between groups used nonparametric criteria - U-Mann-Whitney test to compare two groups and H Kraskel-Wallis for three or more independent groups followed by post-hawk analysis using Dunn's test for pairwise comparison. All statistical calculations were performed using RStudio v. 1.1.442 and R Commander v.2.4-4. Animals were kept in standard vivarium conditions and experiments were performed in accordance with international ethical principles approved by the General Assembly of the World Medical Association "About the Humane Treatment of Animals" (2000), and according to the decision of the Commission on Bioethics of Danylo Halytskyy Lviv National Medical University (protocol №5 of 24.05.2021).

RESULTS

Bacterioscopic examination of smears from the surface of the teeth in the gingival margin of intact rats noted the same type of microbiota, which mainly detected gram-positive microorganisms *Leptothrix*. Cellular elements were 2-3 epitheliocytes and 3-5 leukocytes in the field of view. Bacteriological analysis of the microbiota indicated a predominance of gram-positive species, in particular *non-hemolytic streptococci* (45.11 ± 5.16 CFU/ml) and opportunistic species such as *α-hemolytic streptococci* (65.44 ± 4.36 CFU/ml), *coagulase-negative staphylococci* (10.33 ± 3.67 CFU/ml), *enterococci* (18.33 ± 2.96 CFU/ml) and gram-negative microorganisms - *enterobacteria*, group *Escherichia coli* (7.00 ± 1.50 CFU/ml).

In order to assess the etiological role of individual representatives of the microbiocenosis in the development of pathological processes in the mucous membrane of the gums, we studied the qualitative and quantitative composition of tooth surface microbiota in the gingival margin of rats after 10 weeks of experimental action of the opioid. To achieve this goal, the experimental animals were regrouped according to the severity of the changes that were visualized in the gingival margin of the oral mucosa. We have identified 4 main stages of the process: I degree_- hyperemia, edema, erosion of the mucous membrane of the gums (11.1%); II degree - edema, erosions, single ulcers of the mucous membrane of the gums, the initial signs of violation of the integrity of the bottom of the gingival sulcus (16.7%); III degree - cyanotic mucosa, ulcers, recession of the gums, deepening of the gingival sulcus (27.8%); Degree IV - cyanosis and purulent-necrotic changes of the gingival mucosa, progressive deepening of the gingival sulcus (44.4%).

Table I. Qualitative and quantitative composition of the microbiota of tooth surface in the gingival margin of rats, depending on the severity degree of the pathological process at the end of the tenth week of opioid exposure (CFU/ml).

Bacterial groups	The severity degree of the pathological process			
	I	II	III	IV
α-hemolytic streptococci	52.33±6.40	56.33±8.83	61.56±11.09	61.33±11.22
Coagulase-negative staphylococci	56.33±7.35	65.33±8.50	68.44±7.76*	72.33±8.46*
Escherichia coli	23.22±2.77	24.33±5.98	26.22±8.18	34.44±11.53*
Hemolytic Escherichia coli	1-2	1-2	1-2	1-2
β-hemolytic streptococci	21.22±2.77	39.22±6.72*	43.22±6.28*	48.33±7.04*
Coagulaze-positive staphylococci	9.11±3.86	13.33±3.74	21.33±5.39*	35.44±3.94*, **
Klebsiella	-	-	1-2	9.22±2.05
Pseudomonas aeruginosa	1-2	1-2	4.56±2.65	8.11±1.83***
Yeast-like fungi	4.89±1.54	10.33±2.12*	5.11±1.05**	5.11±1.27***

Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * p<0,05 – significant difference in values relative to the degree I, ** p<0,05 – significant difference in values relative to the degree II, *** p<0,05 – significant difference in values relative to the degree III.

Bacterioscopic examination of smears in animals with degree I revealed a moderate number of leukocytes in the field of view, as well as epitheliocytes, on the surface of which visualized the adsorption of cocci. Degree II animals showed a significant increase in white blood cell count and degeneratively altered epitheliocytes. In degree III, dense accumulations of coccal microbiota, mononuclear cells, erythrocyte autophagocytosis, and gingival epithelial cell degeneration were observed. Destroyed epitheliocytes, segmental leukocytes, erythrocyte autophagocytosis, as well as aggregation of gram-negative bacteria and gram-positive coccal microbiota were visualized in animals with degree IV smears.

In bacteriological studies, attention was paid to microbial pathogens of purulent-inflammatory process, in particular, *coagulase-positive staphylococci* and *β-hemolytic streptococci*, because *coagulase-positive staphylococci* are characterized by the production of toxins with necrotic and hemolytic action (hemolysins, lecithinase), and *β-hemolytic streptococci* produce hemolysin and protein M, which is associated with antiphagocytic and cytotoxic effects on neutrophils. In particular, the number of *coagulase-positive staphylococci* in degree III significantly increased 2.3 times (21.33 ± 5.39 CFU/ml), and in degree IV - 3.9 times (35.44 ± 3.94 CFU/ml), when compared with the I degree of the process (p <0,05). The amount of *β-hemolytic streptococci* significantly increased in degree II - 1.8 times (39.22 ± 6.72 CFU/ml), in degree III of the process - 2.0 times (43.22 ± 6.28 CFU/ml) and at degree IV significantly increased - 2.3 times (48.33 ± 7.04 CFU/ml), compared with degree I of the process in animals with long-term opioid action (p <0,05) (Table I). Such data can be regarded as evidence of a probable etiological factor, namely, these microorganisms in the development and progression of the inflammatory process in the constituent components of the gums.

According to the deepening of pathological changes in the mucous membrane of the gums of rats, the dynamics of growth in the quantitative composition of opportunistic species of microorganisms was also noted (Fig. 1). Among the bacteria we isolated, *coagulase-negative staphylococci* were found to predominate, which do not produce the enzyme plasmacoagulase and are part of common normobiocenoses, in particular, bacterial species *Staphylococcus edermidis*, *Staphylococcus saprophyticus* and others. Thus, the amount of *Coagulase-negative staphylococci* in degree III significantly increased 1.2 times (68.44 ± 7.76 CFU/ml), and in degree IV - 1.3 times (72.33 ± 8.46 CFU/ml) compared with the I degree of the process (p <0,05). At the same time, the amount of *Escherichia coli* (34.44 ± 11.53 CFU/ml) significantly increased 1.5 times in degree IV, compared with degree I (p <0,05).

Due to the fact that the number of *α-hemolytic streptococci* did not change significantly, however, there was a moderate tendency to increase in III (61.56 ± 11.09 CFU/ml) and IV (61.33 ± 11.22 CFU/ml) degree of development of the process, compared with degree I (52.33 ± 6.40 CFU/ml). However, the obtained data on the quantitative composition of *α-hemolytic streptococci* showed a slow and dynamic increase in the number of microorganisms, that are part of the biopellicle with odontopathogenic properties, in particular, the species *Streptococcus* mutans. The quantitative composition of yeast-like fungi, where the species *Candida* albicans predominated, had stable indicators, except for rats with degree II severity of the process, where their number increased significantly 2.1 times, compared with other degrees of severity of the process (p <0,05).

There was also a significant increase in the number of colonies of *Pseudomonas aeruginosa* by 1.8 times in degree IV, compared with degree III (p <0,05). It should be noted that *Pseudomonas aeruginosa* particularly affects the body with a weakened immune system, produces a number of toxins and enzymes that are aggressive factors in the development of sepsis and purulent-inflammatory complications. There was also a significant increase in the quantitative composition of *Klebsiella* (9.22 ± 2.05 CFU/

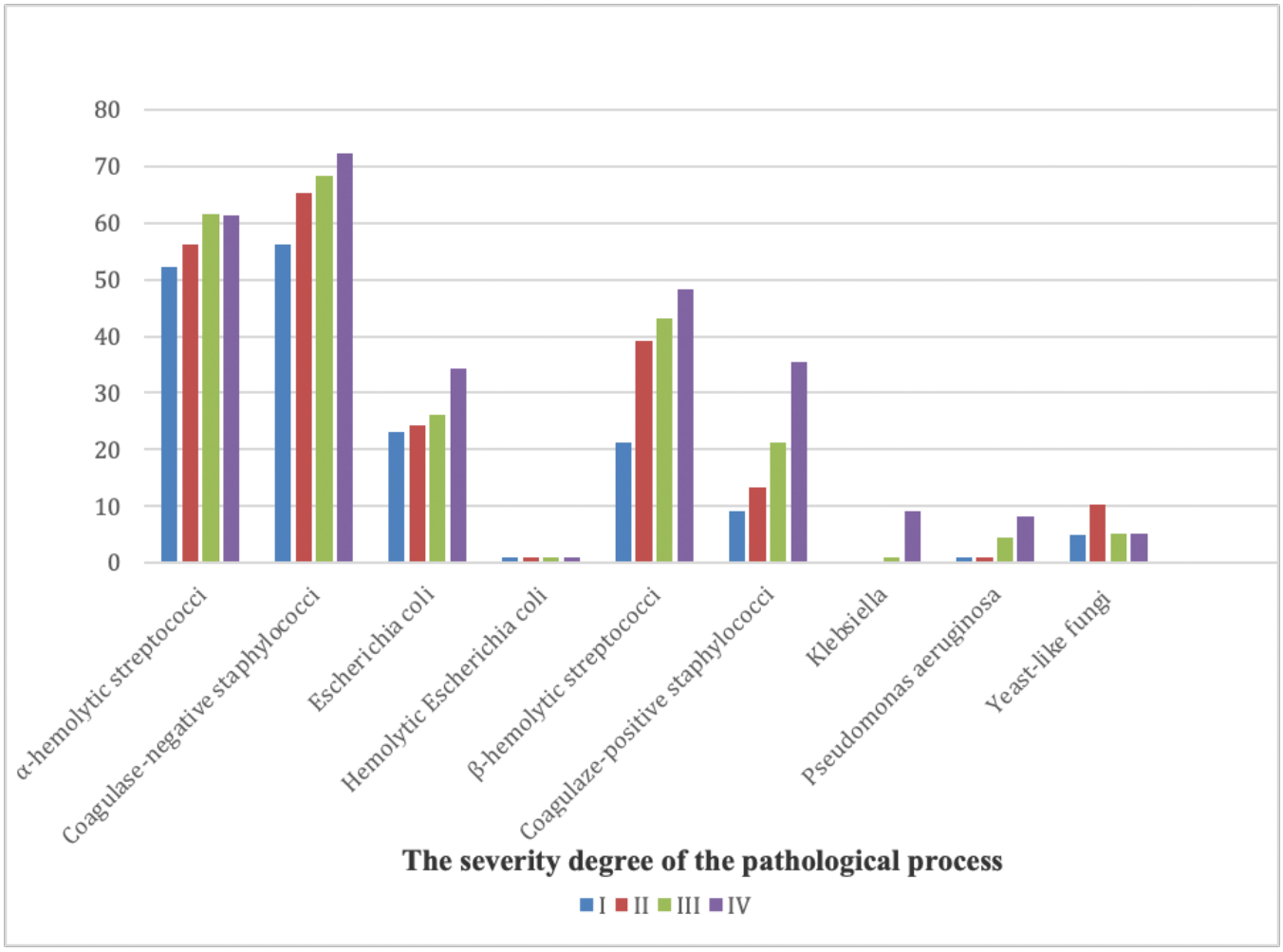

Fig. 1. Dynamics of quantitative indicators of microbial groups at different degrees of severity of the pathological process in the gingival margin under the action of opioids for 10 weeks (CFU/ml).

ml) at degree IV of the process, compared with degree III (1-2 colonies) and not sown in animals at degree I and II of the process. A significant increase in the number of *Klebsiella* colonies in degree IV inflammation indicated the development of dysbiosis involving these capsular bacteria, which have adhesive and antiphagocytic properties, which can provoke the formation of purulent foci and sepsis in the longer term opioid exposure. The number of single colonies of *Hemolytic Escherichia coli* with pathogenic properties was insignificant (1-2 colonies), however, their presence at all stages of the process indicated the stability of dysbiotic changes in the oral cavity of rats under the ten-week action of the opioid.

DISCUSSION

Our results of microbiological studies with experimental opioid exposure can be compared with the results of studies obtained in patients at different stages of periodontitis, where an important role, according to researchers, is played by microbial associations in the deep gingival sulcus, which cause immunoinflammatory reactions that affects the severity of the disease [16, 19]. Besides, the ecological diversity of the periodontal environment may create certain conditions for the colonization of bacterial species, which are usually not considered resident microbiota of the oral cavity and biopellicle are a source of development and spread of systemic infections due to different groups of pathogenic microorganisms [17, 24-26].

However, without clear evidence of the etiotropicity of a particular microorganism to the development of pathological changes in the components of the periodontium, we can assume that only the "main" microbial pathogens cause progression of inflammation in the periodontium, in particular mucous membrane of the gums and oral cavity in general. This is confirmed by the fact that anaerobic bacteria may be one of the main etiological factors of periodontal disease, however, the ecology of these pathogens in the development of the pathological process is inextricably linked with other destabilizing microorganisms of the oral cavity, including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus sanguis* [17-19].

It should also be noted that the microbial spectrum in the oral mucosa of addicted patients is reflected in the qualitative diversity, where a number of researchers have noted the

predominance of coccal, anaerobic, fungal microbiota and *Klebsiella* [12, 14, 27]. All these bacterial groups were also detected at 10 weeks of opioid action, which indicated the etiological role of these microorganisms in the progression of the inflammatory process in the paradentium. Our research suggests that there is a relationship between several microbial associations and pathomorphological changes in the paradentium area, based on which we identified 4 degrees of pathological process in the mucous membrane of the gums of rats under the ten-week action of the opioid.

CONCLUSIONS

The relationship between quantitative and qualitative characteristics of the microbiocenosis of the tooth surface in the gingival margin and the severity of changes in the mucous membrane of rat gums after the ten-week action of the opioid was determined and this fact indicates bacterial associations of dental biopellicle as an etiological factor in the development of the inflammatory process and reflected the state of the oral microbiome in general.

Depending on the severity of the pathological process in the mucous membrane of the gums of rats with long-term action of opioids, a significant increase in the quantitative indicators of bacterial pathogenic *(β-hemolytic streptococci, coagulase-positive staphylococci)* and opportunistic pathogens microbiota (*coagulase-negative staphylococci, Escherichia colli*) at IV degree of development of process in comparison with I degree was determined. The appearance of potential pathogens - *Klebsiella* and *Pseudomonas aeruginosa* at degree IV of the process indicated profound dysbiotic changes, which in combination with other microbial associations indicated a dynamic progression of purulent-inflammatory process in the mucous membrane of the gums.

The identified changes in the qualitative and quantitative composition of microbiota of biopellicle reflect the etiopathogenesis of inflammatory diseases of the oral cavity and may serve as criteria for differential diagnosis of periodontitis in opioid-dependent individuals.

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ORIGINAL ARTICLE

DISTRIBUTION OF INTEGRON III AND PHYLOGENIC CLADE AMONG MDR UROPATHOGENIC *E. COLI* FROM PATIENT IN AL-DIWANIYAH CITY, IRAQ

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ABSTRACT

The aim: The goal of this study is to identify Uropathogenic *E. coli* multidrug-resistant bacteria, as well as their genetic profile clade and correlation with dispersion integron.

Materials and methods: Five hundred urine samples from UTI patients were collected in Al-Diwaniyah, Iraq. They were then investigated by a qualified consulting doctor. After cultivation in special media (MacConkey agar and Eosin-Methylene blue) to detect Enterobacteriacea, including *Escherichia coli*, the samples were identified using the Vitec 2 compact system, as well as MIC susceptibility testing to Amikacin, Levofloxacin, Cefepime, Meropenem, Nitrofurantion, and Trimethoprim-sulfamethoxazole for detect multidrug resistance isolates. Multiplex PCR was used to detect three types of integrase gene. Finally, ERIC2-PCR was used to detect the genetic profile of all isolates.

Results: From 500 UTI samples, 22 isolates UPEC detected resistance to different class of antibiotic, including: 86.3% to Cefepime, 54.5% - to Trimethoprim-sulfamethoxazole, 31.8% -Levofloxacin, 18.8% - Amikacin, 18.8% - Imipenem, 0% to Nitrofurantion. Twenty of the 22 isolates had various integrase gene classes as: 54.5%, 36.3%, 72.2% for Intl I, Intl II, and Intl III, respectively; while two isolates have no integron.

Conclusions: Integron III has a higher incidence and compensates other classes; isolates with triple classes are more virulent and antibiotic resistant. Their genomic profile reveals association with human urine and unique clade of relatives.

KEY WORDS: integron III, phylogenic clade, MDR uropathogenic

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INTRODUCTION

Rising antimicrobial resistance in microorganisms has been related to uncontrolled abuse of antibiotics during therapy and prophylaxis that lead to appear new resistance strain to multi –drug (MDR) uropathogen *E.coli* [1,2]thus providing a further impetus for search for exploring new therapeutic measures. In this direction, bacteriophages, commonly called phages, are recently being considered as potential alternatives for treatment of UPECs. Phages are the tiniest form of viruses which are ubiquitous in nature and highly specific for their host. This review discusses the possible ways of using natural phages, genetically engineered phages, and phage lytic enzymes (PLEs, which is a serious global public health concern [3]. Multi drug resistant strains of *Escherichia coli* (*E. coli*) can be found in both human and animal isolates worldwide [4-5] oxytetracycline (OT with multiple drug resistant non-pathogenic *E. coli* found in the intestine, implicated as important reservoir of resistance genes [6]. The management of intra and extra intestinal infections caused by *E. coli,* which are a major source of illnesses, death, and increased production costs, is complicated by acquired multi-drug resistance to antimicrobial medicines [7]. Horizontal gene transfer of mobile genetic elements like integron, which has been known to associate resistance genes, could allow pathogenic *E. coli* strains to acquire resistance genes. Integrons, which can contain genes for sit-specific recombination and are capable of grabbing and mobilizing gene cassettes, are known to be carried by mobile genetic elements such as plasmids and transposes [8]. Integron contains two common characteristics, a structural part and an array of gene cassettes (GCs). The 5'-conserved region (5'C.S) consists of an integron integrase (*intI*) gene, an integron-associated recombination site (attI), and an integron-associated promoter (Pc) site. A site-specific tyrosine recombines enzyme called integron integrase, encoded by the *intI* gene, which has a specific activity of integration and excision of GCs. The expression of integrase genes can be induced by accumulation of single-stranded DNA in a bacterial cell via conjugation, transformation, starvation, and/or exposure to antibiotics [9-10].

Integrons are categorized into types: the super-integron and the antibiotic resistance integron (ARIs) [11]. Dissemination of antibiotic resistance genes among bacteria can occur by mobile genetic elements containing the ARIs [8]. There are four classes of integron (classes 1, 2, 3 and 4), which are known to carry multi-drug resistance genes [12]. Class 1 integron is the most widespread and has been frequently found in ESBL producing clinical isolates of *Enterobacteriacea [13]47 per cent (9/19*. Class 2 integron occurs less frequently in *E.*

Table I. Specific primer use to detect IntI,II,III and ERIC

Integron class		Primer sequences (5˚ _ 3˚)	Product size	Reference
IntI1	F	TCT CGG GTA ACA TCA AGG	254 bp	[16]"Int1", "Int2", "Int3", "prevalence", "antibiotic resistance", "Klebsiella pneumoniae", and "Iran, with the aid of Boolean operators ("AND" or "OR"
	R	AGG AGA TCC GAA GAC CTC		
IntI2	F	CAC GGA TAT GCG ACA AAA AGG	788bp	
	R	TGT AGC AAA CGA GTG ACG AAA TG		
IntI3	F	AGT GGG TGG CGA ATG AGT G	variable	
	R	TGT TCT TGT ATC GGC AGG TG		
ERIC	1	ATG TAA GCT CCC TGG GG AT TCAC	variable	
	2	AAG TAA GTA ACT GGG GTA AGCG		

Table II. Prevalence of Integrase gene classes

No. Isolate	IntI1	IntI2	IntI 3	Resist antibiotic						
				MDR	lev	AK	FEP	IMI	F	STX
U1	250	700	1300	MDR	-	-	+	+	-	-
U2	250	----	200	MDR	-	-	+	-	-	+
U3	250	700	500	MDR	.	-	+	-	-	+
U4	250	700	800	MDR	.	-	+	-	-	+
U5	250	700	----	MDR	-	-	+	-	-	-
U6	250	700	800	MDR	-	-	+	-	-	-
U11	250	600	100/200	MDR	-	-	+	-	-	-
U12	250	600	100/300	MDR	-	-	+		-	-
U13	250	-----	1200	MDR	+	+	+	+	-	+
U14	------	-----	800	MDR	+	-	+	+	-	-
U16	250	-----	900	MDR	-	-	+	-	-	+
U17	------	-----	500	MDR	+	+	-	+	-	-
U18	-------	-----	900	MDR	+	+	+	-	-	+
U19	------	-----	500	MDR	+		+	-	-	+
U20	------	----	400	MDR	+	-	+	-	-	+
U21	-----	700	900	MDR	-	-	-		-	-
U22	----	----	900	MDR	-	-	+		-	-
U25	250	----	---	MDR	-	-	+	-	-	+
U26	250	-----	----	MDR	-	-	+	-	-	+
U39	-----	-----	400	MDR	-	-	-	-	-	+
U40	-----	-----	----	MDR	-	-	+	-	-	-
U43	-----	-----	----	MDR	+	-	+	-	-	+
total	12	8	17	22	7	4	19	4	0	12
%	54.5%	36.3%	77.2%		31.8	18.8%	86.3%	18.8%	0%	54.5%

coli and *Klebsiella pneumoniae,* while class 3 integron is rarely found [14]. Class 4 integron has only been described in *Vibrio cholera* strain [15]. This study looked into the prevalence, distribution, and phylogenicity of classes 1, 2, and 3 integron in multi-drug resistant *E. coli* recovered from patients.

THE AIM

The goal of this study is to identify Uropathogenic *E. coli* multidrug-resistant bacteria, as well as their genetic profile clade and correlation with dispersion integron.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 500UTI urine samples from patients were examined by consultant at Al-Hamza Hospital and in two private clinics, in Diwaniya province, Iraq, from October 2020 to February 2021. Each urine sample was collected from patient into a sterile container. The sample was streaked on the blood agar, EMB agar, MacConkey agar using standard loop method. The media were incubated at 35 ºC for 24 hours.

Table III. Integron Triple- Existence

isolate	Intl I	Intl II	Intl III
U1	250	700	1300
U3	250	700	500
U4	250	700	800
U6	250	700	800
U11	250	600	100/200
U12	250	600	100/300
Total	6/22	6/22	6/22
%	27.2 %	27.2 %	27.2 %

Table IV. Integron Single existence

isolate	Intl I	Intl II	Intl III
U14	------	-----	800
U17	------	-----	500
U18	-------	-----	900
U19	------	-----	500
U20	------	----	400
U22	----	----	900
U39	-----	-----	400
U25	250	----	---
U26	250	-----	----
TOTAL	2/22	0/22	7/22
%	9.0 %	0%	31.8 %

Table V. Integrons Co-existence Intl I, Intl II

isolate	Intl I	Intl II
U5	250	700
TOTAL	1/22	1/22
%	4.5 %	4.5 %

Table VI. Integron Co-Existence Intl I, Intl III

isolate	Intl I	Intl III
U2	250	200
U13	250	1200
U16	250	900
TOTAL	3/22	3/22
%	13.6%	13.6 %

BACTERIAL ISOLATION AND BIOCHEMICAL TESTS FOR THE IDENTIFICATION OF *E. COLI*

Examination of the urine samples was completed within 24 h after collection. They were inoculated onto MacConkey agar and positive one subculture on Eosin-Methylene blue (EMB) plates. After 24 h incubation at 35 ± 0.5°C for 24 ± 2 h, the gram-negative microorganisms were isolated from MacConkey and EMB agar and determined at the species level, using cytochrome oxidase, triple sugar iron agar, urea and Indole tests as putatively *E. coli*. All suspicious isolates were biochemically identified by Vitec 2 compact, an excellent tool for accurate identification.

Table VII. Integron Co-Existence Intl II, Intl III

isolate	Intl II	Intl III
U21	700	900
TOTAL	1/22	1/22
%	4.5 %	4.5 %

Table VIII. Distribution of Integrons

Integrons distribution	No isolate
single	9
Co	5
triple	6

Table IX. EIRC-2-PCR clade of 22 clinical Uropathogenic strains

Strain	Profile number or clade	Prevalence %
Ec14, Ec16, Ec18, Ec19	P2 or clade 2	18%
Ec6,	P4 or clade 4	4.5%
Ec5, Ec8	P6 or clade 6	9%
Ec14	P7 or clade 7	4.5%
Ec1, Ec4	P8 or clade 8	9%
Ec24, Ec22	P9 or clade 9	9%
Ec25, Ec26,	P10 or clade 10	9%
Ec40, Ec43	P11 or clade 11	9%
Ec4, Ec32	P12 or clade 12	9%
Ec3	P13 or clade 13	4.5%
Ec39	P14 or clade 14	4.5%
Ec2,	P16 or clade 16	4.5%

NOTE: The color black denotes a urine source, the color orange denotes a distinct source, and the color blue denotes an isolate that lacks the integrase gene.

DNA EXTRACTION AND POLYMERASE CHAIN REACTION (PCR) CONDITIONS FOR DETECTION OF INTEGRON

Bosphore® was used to extract DNA from bacteria kept in broth Bacterial DNA Extraction Spin Kit (Anatolia, Turkey). These methods are based on the silica membrane column separation: to get nucleic acids they must first remove and purify bacterial nucleic acids. The process consists of three steps: lysis, DNA binding, and eluting. The PCR reactions were performed in sterile 0.2 ml PCR tube sitting on ice, with forward adding and revering primer integron, table (1); 3µl, Taqmastermix with dye 12.5, temple DNA 5 µl and complete the size to 25µl with Nuclease- free water were also used.

PCR REACTION

After preparing of mixture, we incubated the tube in a thermal cycler at 94°C for 3 min to completely denature the template. In this study we performed 30 – 35 cycles of PCR amplification as: denaturation, 94°C for 30 sec;

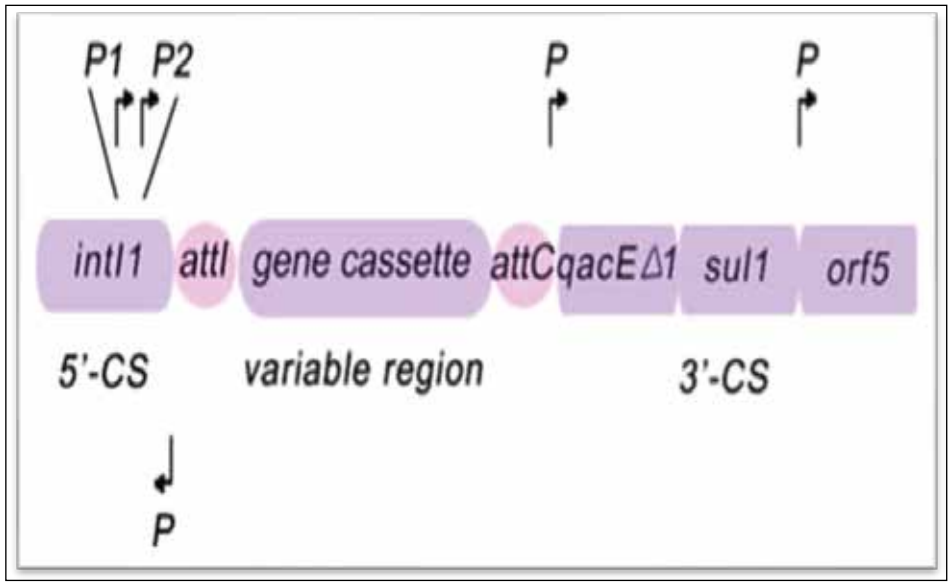

Fig 1. Integron structure

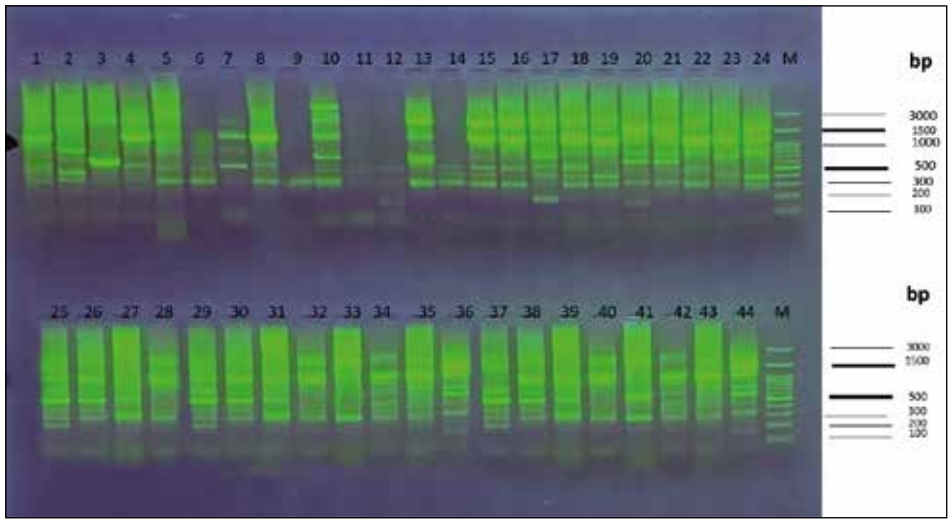

Fig 2. Safe- green Agarose gel electrophoresis (1%) showing ERIC-2 –PCR banding pattern for *E. coli* clinical strains

Annealing: 45-72 for 30 sec; Extending: 72°C for 1 min/1 kb template. Then the tube was incubated at 72°C for five min and maintained the reaction at 4 °C, then the sample was stored at -20°C for other investigation.

GEL ELECTROPHORESIS

We analyzed the amplification product by 1.5 agarose gel electrophoresis and visualized it by dye added with master mix read the move by UV, using 20 µl molecular weight.

PCR CONDITIONS FOR DETECTION ENTEROBACTERIAL REPETITIVE INTERGENIC CONSENSUS-PCR

The molecular fingerprint of the enterobacterial repetitive intergenic consensus (ERIC) region was studied via PCR. The PCR primers employed were show in specific primer table I. Each reaction was conducted at a final volume of 50 µL and consisted of 0.4 mol/m3 of primers, 0.05 mol/m3 dNTP mix, 0.025 U/µL Taq DNA Polymerase, 21 mol/m3 MgCl2, 10 µL of Colorless Buffer (Promega, USA), and 32.75 µL of ultrapure H2O. The samples were run-in a MULTIGENE Lab net themocycler with the following program: 95 °C for 7 minutes; 30 cycles of 94 °C for 1 minute, 52°C for 1 minute, 65°C for 8 minutes; and a final extension at 65 °C for 15 minutes. The amplicon was inspected in electrophoresis in 1 % agarose gels-(1X TAE); the 1 kb DNA ladder (Promega) was used as a molecular marker, and Gel Red (Biotium, USA) was used as an intercalator. The run conditions were 70 V for 90 minutes in a Multisub Electrophoresis System Cleaver Scientific chamber. The gel was visualized in a Smart Doc Imaging Enclosure Benchmark Accuris E300 UV photo documentation system at a wavelength of 302 nm. Subsequently, the polymorphisms were analyzed with the NTSYS Spc 2.1 software (License UH3071IX).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility tests were performed by MIC method using Use AST-GN76 card. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acid-

ification, alkalization, and enzyme hydrolysis according to the Clinical and Laboratory Standards Institute guidelines. After incubating the inoculated plate aerobically at 37°C for 18–24 h in an aerobic atmosphere, the susceptibility of the *E. coli* isolates to 6 antimicrobial agents was determined, and the results were interpreted in accordance with interpretive criteria provided by CLSI. *E. coli* ATCC®25922™ and ATCC®35218™ was used as quality control organisms in antimicrobial susceptibility determination. The following antibiotic MIC were used: Cefepime (FEP) with concentration of 2, 8, 16, 32 mg/ml; Imipenem (IMI) - 1, 2, 6, 12 mg/ml; Amikacin (AN) - 8, 16, 64 μg/ml; Levofloxacin (LEV) - 0.25, 0.5, 2, 8 μg/ml; Nitrofurantoin F - 16, 32, 64 μg/ml; Trimethoprim/ Sulfamethoxazole (SXT) - 1/19, 4/76, 16/304 μg/ml.

RESULTS

Out of the 500 UTI samples, 22 harbored *E. coli* after culture and biochemical tests by Vitec 2 compact. Hence the prevalence of *E. coli* in UTI sample was 4.4%.

ANTIMICROBIAL RESISTANCE

All isolates exhibited multi-drug resistance phenotypically. They were most resistant to Cefepime 86.3%, 54.5% - to Trimethoprim-sulfamethoxazole, 31.8% - to Levofloxacin, 18.8% to Amikacin, 18.8% to Imipenem and were no resistant to Nitrofurantion (0%). (Table I). A surprisingly high increase was observed in the resistance of *E. coli* to antimicrobial agents from 2014 to 2018 [17]. In Zakho City, Iraq, Imipenem was the most effective antibiotic, with only 2.84 percent of patients developing resistance [18]El-Behera and Damietta. In Diyala, Iraq the resistance to Imipenem was 26.04% with high resistance to other antibiotic [19]. Thus, the average resistance varies in different areas.

DETECTION OF INTEGRON

Out of these *E. coli* isolates, 20 (90%) carried integron, 12 (54.5%) isolates carried Class 1 integron, 8 (36.3%) isolates carried class 2 integron, and 17 (77.2%) isolates carried class 3. (Table II). In Diwaniyah city, Iraq, the Integrase III gene is prevalent to other Enterobacteriacea [20]. All integron of clinical importance classes 1, 2 and 3 have inserted into transposable elements, therefore, markedly increasing their potential mobility [21]. The clinical class 1 integrons immediate ancestor, for example, is assumed to be formed when a chromosomal integron from an environmental betaproteobacterium was introduced into a transposing [22]. The proteobacteria organisms capable of living in low-nutrient environments such as deep oceanic sediments, glacial ice, or deep undersurface soil [23]. As a result, contamination of water, vegetables, and animals plays a part in Integron dissemination.

INTEGRON DISTRIBUTION

All Integron-containing isolates have a varied distribution, such as single existence, co-existence, or triple existence. Nine isolates have a single existence, five isolates have co-existence, and six isolates were triple isolates, out of a total of 22 isolates, table VIII.

MOLECULAR PROFILE WITH ERIC2-PCR

The ERIC-2-PCR banding pattern demonstrated larger number of DNA bands. The most abundant DNA bands were 1500, 1000, 500, 400, and 300 bp. However, the least abundant DNA bands were 3000, 2800, 2000, and 100 bp figure 2.

The upper panel and the lower panel included the ERIC-1-PCR banding pattern for clinical strains from Ec1 to Ec24, M: 100 bp DNA ladder. According to the ERIC-2-PCR banding pattern, the twenty- two clinical strains of *E. coli* could be categorized in 12 profiles or clade, (table IX); 60% of clinical isolates were distributed throughout 12 clades, whereas the remaining isolates were not clustered into any clade. The Clade 2 was the most prevalent - 18%, followed by Clade 6, 8, 9, 10, 11, 12 (9%), and Clade 4, 7, 13, 14, 16 (4.5%).

DISCUSSION

According to the findings obtained, ERIC2-PCR had a higher discrimination performance, in comparison to finding by Mahmoud, in Egypt (2020): assigning of 42 isolates into three clade may indicate the dissemination of distinct clonal groups that may be responsible for Hospital-acquired infection with UPEC isolates [24]. In Iraq, Merza and Jubrael mentioned patterns analysis where 98 UPEC strains were divided into a few clusters only two major cluster groups [25]. In contrast, another study reported different patterns of analysis, such as by Ramazanzadeh et al. who found that ERIC-PCR differentiated *E. coli* isolates into a higher number of cluster groups (20) and suggested that these isolates were not clonally spread and resulted from individual infections, which may be due to the low rate of hospital infection in this area [26]. The finding shows in the table 3, the isolate in clade 2 is the most inclusive with similarity (18.0%). UPEC isolates are found in clade 2, 4, and 8. Clade 6, 9, 10, 11, 12, 14, 16, comprise mix isolate from urine and stool sources with very few similarities (9.0%) near Hakimi, Iran (2019). A total of 21 ERIC-PCR profiles were found among all of the isolates, that included 12 profiles among human isolates and 10 profiles among swimming pools isolates, and there was one similar profile between them. The existence of similar characteristics in both sources suggests that these isolates may rotate between human-water-human [27]. There is no integron in bacterial DNA in clade 11 isolates. As a result, the clade with the highest incidence is related to urine source and has integron III, while Dawood, (2020), Diyala, Iraq, concluded that there is a great genetic variability among the isolates, only 3 pairs of clones showed 100% homology, in about 16 isolates there was homology greater than 80%, with the remaining isolates showing lower homology, and associated with integrase gene class I, their obtained suggest an inversion in the flow of MDR strains from the community into the hospital environment

[28]such as plasmids and transposons have recently been shown, to promote the exchange of genetic material between genera and bacterial organisms. The purpose of this research was to investigate the prevalence and diffusion of resistance genes in the sample community (n=100.

The isolates Ec14 and Ec3 were unique to the clade 7 and 13. Isolates showed unique banding patterns indicating genetic un-relatedness, whereas the others were related to the common clade. Sharefi, (2020), in Iran, showed two clonal complexes with 71 isolates and only nine isolates were stayed out of clonal complexes in the form of a unique, as well as Sabri and Kareem, Iraq, Bagdad, (2020) find that from 50 *E. coli* isolates subjected to ERIC-PCR, 47 had amplications ranging from 700 to < 3000bp, the generated banding profile showed highly polymorphic DNA fragments in these 47 isolates, while three isolates were untypical [29]. There were also four isolates from clade 2 that had the triple integrase gene and were resistant to cephalosporin and Trimethoprim-sulfamethoxazole. The number of isolates was 14, 16, 18, and 19. Isolates with the Integrase gene type III, which has a multiband and high molecular weight, have been linked to the emergence of Imipenem resistance, table 2. Clade 6, c9, c10, c11, c12, and c16 are among the clade with isolates from a variety of sources. Clade 6, 9, 10, 11, 12, 14, 16 were the unique clades found in the entire variety source, while Clade 2, 8 were mainly associated to human urine isolates.

As a result, personal hygiene is critical in the transmission of this multidrug-resistant *E. coli*. The higher rate of resistance to several classes of antibiotics in integron-positive isolates is probably attributable to the association of resistance genes with integron-carrying on both chromosomal and plasmids and hence suggests that antibiotic drug resistance is transmitting through these mobilizing agents [30]such as Orf513, are being increasingly linked to mega-antibiotic-resistant regions. While their overall nucleotide sequences show little identity to other mobile elements, amino acid alignments indicate that they possess the key motifs of IS91-like elements, which have been linked to the mobility ent plasmids in pathogenic Escherichia coll Further inspection reveals that they possess an IS91-like origin of replication and termination sites (terIS. The isolates preformed ERIC2 -PCR show high genetic diversity may be responsible for MDR *E. coli*. The isolates U5, U2, and U4 were resistant to Minocycline, an antibiotic that is not used in UTI infections caused by *E. coli* (according to CLSI), and the isolates from the clade were mixed, that is to say, the acquired from a lineage. There were also four isolates from clade 2 that had the triple integrase gene and were resistant to cephalosporin and Trimethoprim-sulfamethoxazole. The number of isolates was 14, 16, 18, and 19. The isolates having the integrase gene type III with multiband and high molecular weight have been associated to emerge Imipenem resistance, Table 2. As evident from RAPD-typing, most patients were infected with different clade of organisms, thereby demonstrating clonal diversity among isolates suggesting horizontal transmission of resistance genes [31].

CONCLUSIONS

The increased incidence of Integron III in isolates with triple existence means that antibiotic resistance is more likely to develop. And because the phylogenic of integrase gene type III is linked to proteobacteria and environmental dissemination, pollution from animals and their products, state of plants, soil, and water must be controlled to prevent transmission to humans. Otherwise, public health officials must devise a strategy to combat emerging antibiotic resistance and develop new treatment protocols for urinary tract infections.

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Conflict of interest:
The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

EMOTIONAL IMPACT OF THE COVID-19 PANDEMIC ON HEALTHCARE WORKERS IN UKRAINE

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ABSTRACT

The aim: To study the emotional impact of the COVID-19 pandemic on healthcare workers in Ukraine.

Materials and methods: 1087 healthcare workers across all regions of Ukraine completed an online questionnaire. Respondents were divided into two groups: A – 863 (79.4%), B – 224 (20.6%), according to whether or not they experienced anxiety/fear related to COVID-19.

Results: Such factors as risk of contracting COVID-19, news of new cases, insufficient staff in healthcare facilities, risk of transmission of COVID-19 to family or friends, risk of death from COVID-19, the threat of the spread of the COVID-19 pandemic were associated with high level of anxiety/fear among healthcare workers. Factors that facilitate coping with stress included absence of Covid-19 cases among staff, recovering patients, availability of gear and protective equipment and absence of COVID-19 infected among loved ones, relieving stress (relaxation, going info sports and so on), communication with family and friends to relieve stress and get support, avoidance of COVID-19 media reports in particular on deaths connected cases, getting information about the spread of Covid-19 only from legitimate sources.

Conclusions: The data obtained in this study indicate an urgent need for the development and implementation of preventive and rehabilitation measures aimed at stabilizing the psycho-emotional state of health workers and improving the quality of care in the special conditions of the COVID-19 pandemic. Achieving these goals will be facilitated by taking into account the specifics of the response to COVID-19 and measures to reduce the effects of stress while enhancing the effects of stressors among health care workers.

KEY WORDS: COVID-19 pandemic, healthcare workers, stress, fear, anxiety

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INTRODUCTION

Existing research shows that COVID-19 pandemic has caused a huge mental health burden on the population, especially on healthcare workers who are facing high risks and increased workload. The perceived stress has a negative impact upon both the mental and physical health of the healthcare workers [1]. Stress symptoms in healthcare workers were significantly more pronounced during versus before COVID-19. For example, for hypervigilance, there was 12-fold odds during versus before COVID-19 [2]. Healthcare workers showed more anxiety and depression symptoms compared with the general population [3-5]. Medical workers experienced high prevalence of stress, anxiety, depression, insomnia, COVID-related traumatic experiences, somatization, and obsessive-compulsive symptoms [6-10]. Some research shows that healthcare professionals working on the frontline had significantly higher levels of PTSD symptoms and depression compared to those working indirectly [8, 9]. But others state no significant link between direct contact with patients with COVID-19 at work and anxiety, depression, or stress [10]. Risk factors for stress-related symptoms included living in rural areas, being female, being a nurse, being younger age, being directly engaged with COVID-19, having pre-existing psychological problems, working all night for >3 days, concerns about infection at work, fear at work, perceive troubles at work, having a colleague deceased, hospitalized or in quarantine, negative metacognitions, burnout, health anxiety and lack of emotional support, uncertainty about when the epidemic will be under control [6-9, 11, 12].

A lot of European countries conducted cross-sectional surveys [10], but there is no data from Ukraine.

THE AIM

The aim of the present study was to analyze the emotional status among the health care workers in Ukraine while they were facing with Covid-19 depending on their sociodemographic characteristics and different stress–related factors.

MATERIALS AND METHODS

1087 healthcare workers across all regions of Ukraine completed an online questionnaire. The questionnaire

Table I. Comparative characteristics of respondents in groups A and B by sociodemographic parameters

Sociodemographic parameters	Groups of respondents		Number of answers	Statistical significance		
	A[a] n (%)	B[b] n (%)	N	χ^2 [c]	df[d]	p[e]
Gender						
Female	682 (78.8)	183 (21.2)	865	0.68	1	0. 408
Male	175 (81.4)	40 (18.7)	215			
Age						
Under 30 years	139 (78.1)	39 (21.9)	178	11.57	4	0.020
31-40 years	203 (77.5)	59 (22.5)	262			
41-50 years	235 (83.9)	45 (16.1)	280			
51-60 years	200 (81.3)	46 (18.7)	246			
Over 60 years	80 (69.6)	35 (30.4)	115			
Marital status						
unmarried	151 (81.2)	35 (18.8)	186	0.93	3	0.818
married	575 (78.7)	156 (21.3)	731			
divorced	102 (81)	24 (19.1)	126			
widow/ widower	30 (76.9)	9 (23.1)	39			
Presence of minor children						
present	529 (79.2)	139 (20.8)	668	0.01	1	0.912
absent	329 (79.5)	85 (20.5)	414			
Live						
with husband/wife	552 (79.2)	145 (20.8)	697	2.98	3	0.393
with parents	75 (74.3)	26 (25.7)	101			
alone	171 (80.7)	41 (19.3)	212			
other	60 (84.5)	11 (15.5)	71			
Health care institution						
Centers for primary health care	152 (77.2)	45 (22.8)	197	5.89	5	0.317
Family medicine clinics	115 (80.4)	28 (19.6)	143			
City multidisciplinary hospitals	103 (76.3)	32 (23.7)	135			
Central district hospitals	102 (75)	34 (25)	136			
Regional hospitals	113 (84.3)	21 (15.7)	134			
Others	275 (81.4)	63 (18.6)	338			
Occupation						
Chief of the clinic	24 (77.4)	7 (22.6)	31	4.28	5	0.509
Chief of department	131 (83.4)	26 (16.6)	157			
Doctor	496 (79)	132 (21)	628			
Nurse	109 (76.2)	34 (23.8)	143			
Psychologist	14 (70)	6 (30)	20			
Others	85 (82.5)	18 (17.5)	103			

Specialty						
Family medicine	177 (77.6)	51 (22.4)	228	11.33	12	0.500
Therapist	92 (81.4)	21 (18.6)	113			
Pediatrics	59 (84.3)	11 (15.7)	70			
Infectious diseases	18 (78.3)	5 (21.7)	23			
Surgery	23 (85.2)	4 (14.8)	27			
Psychiatry	144 (84.2)	27 (15.8)	171			
Addiction	9 (81.8)	2 (18.2)	11			
Cardiology	32 (74.4)	11 (25.6)	43			
Intensive Care Medicine	17 (65.4)	9 (34.6)	26			
Obstetrics and gynecology	14 (82.4)	3 (17.7)	17			
Other	221 (77.3)	65 (22.7)	286			
Department						
Outpatient clinic	154 (76.2)	48 (23.8)	202	10.88	9	0.283
Intensive care unit	32 (69.6)	14 (30.4)	46			
Therapeutic	101 (78.9)	27 (21.1)	128			
Psychiatry clinic	116 (85.3)	20 (14.7)	136			
Infectious	25 (89.3)	3 (10.7)	28			
Surgical	26 (83.9)	5 (16.1)	31			
Pediatrics	45 (79)	12 (21.1)	57			
Emergency medical care	14 (66.7)	7 (33.3)	21			
Administrative	45 (79)	12 (21.1)	57			
Other	282 (79.4)	73 (20.6)	355			

Notes: [a] – group of respondents experiencing anxiety/fear, [b] – group of respondents who are not experiencing anxiety/fear, [c] – Pearson's Chi-Square test, [d] – degrees of freedom, [e] – p-value of significance.

was created taking into consideration the experience and results of the survey conducted during the SARS epidemic in 2003 [13, 14]. The questionnaire included three groups of questions: 1) types of response to the COVID-19 related situation; 2) factors that affect the occurrence of stress; 3) factors that help to overcome stress. Socio-demographic characteristics of health care workers were analyzed. However, not all respondents provided complete answers to all the groups of questions, thus the values for individual categories of questions ranged from 1039 to 1087.

Descriptive statistics were used to illustrate socio-demographic indicators and to distribute estimates of answers to questions. Pearson's criterion $\chi 2$ was applied to compare group differences in categorical variables (socio-demographic characteristics). The nonparametric Mann-Whitney U-test was used to compare the severity of each stress factor in groups of respondents. A $p<0.05$ value was considered statistically significant. The results of the study were processed using the statistical package of the licensed program TIBCO Statistica 13.4 [15, 16].

All participants gave informed consent to participate and the study was approved by the Institutional Ethics Committee.

RESULTS

Psycho-emotional response in the form of pronounced anxiety / fear caused by the COVID-19 pandemic was detected in every fourth of five medical workers - 863 (79.4%), while 224 (20.6%) respondents said they had no or weak severity of such manifestations. Respondents were divided into two groups: group A - those who experienced severe anxiety/fear in connection with COVID-19, group B - all other medical workers.

The relationship between feelings of anxiety/ fear related to COVID-19 with certain socio-demographic parameters in healthcare workers was assessed. The age of the specialists influenced their feelings of anxiety and fear associated with COVID-19 ($p=0.02$). Specialists under the age of 40 declared no difference in the assessment of their fear and

Table II. Differences between groups A and B in the perception of stress-factors (Mann-Whitney U-test)

Factors	Rank Sum		U[c]	p[d]
	Group A[a]	Group B[b]		
The risk to get COVID-19	381524	167104	38318	0.000
The possibility to transmit COVID-19 to relatives, friends	391472	146732	52396	0.000
Absence of information about the exact time the outbreak will be under control	332492	130712	39497	0.000
Constant news about new COVID-19 cases in television / social networks	376634	149192	42481	0.000
Conflict between duty and personal safety	380910	152119	46757	0.000
Physical exhaustion / fatigue	386441	138360	56363	0.000
Irritability due to increased workload	419788	125703	75753	0.000
Complex challenge associated with treatment of COVID-19	371211	142381	49208	0.000
Seeing patients with COVID-19 dying	356346	122385	56421	0.000
Observing colleagues being stressed	379156	144620	48265	0.000
Absence / insufficiency of personal protective equipment	378805	133774	57604	0.000
The need to wear protective equipment on a daily basis	404240	123638	66809	0.000
Lack of staff in your health care institution	368691	129810	53870	0.000
Difficulty getting to work	396606	124104	65715	0.000

Notes: [a] – group respondents experiencing anxiety/fear, [b] – group respondents who is not experiencing anxiety/fear, [c] – Mann–Whitney U statistic, [d] – p-value of significance.

Table III. Differences between groups A and B in perception of COVID-19 related situation (Mann-Whitney U-test)

Factors	Rank Sum		U[c]	p[d]
	Group A[a]	Group B[b]		
Attempts to reduce contact with Covid-19 patients	428028	119553	81500	0.032
Thoughts about quitting the job	408264	136182	65058	0.000
Thoughts about getting a sick leave	406545	130621	68292	0.000
Thoughts about quitting the job if one of your colleagues gets COVID-19	412185	128096	71460	0.000
Thoughts about quitting the job if one of your colleagues dies from COVID-19	410257	133147	67879	0.000
The feeling that employees who did not work with COVID-19 were avoiding you	412609	123522	72709	0.000
The feeling that others were avoiding you because you might have had contact with coronavirus	416085	130451	72050	0.000

Notes: [a] – group respondents experiencing anxiety/fear, [b] – group respondents who is not experiencing anxiety/fear, [c] – Mann–Whitney U statistic, [d] – p-value of significance.

anxiety level related to the pandemic. Specialists between the ages of 41 and 50 demonstrated low level fear/ anxiety, simultaneously specialists over the age of 60 had high levels of fear/ anxiety. No difference was found between other socio-demographic indicators and level of anxiety/ fear related to COVID-19 (p>0.05) (Table I).

Factors that demonstrated the most pronounced association with COVID-19 anxiety / fear were the threat of illness or death of the specialist or his / her relatives or colleagues. In particular, such factors included "high probability to get coronavirus", "the possibility to transmit COVID-19 to relatives, friends" (p = 0.0001). A strong association with anxiety/fear related toCOVID-19 was indicated by the group of factors connected with objective and subjective shortcomings and inconveniences of the organizational process in providing care to patients. In particular, such factors included "absence / insufficiency of personal protective equipment", "the need to wear protective equipment

Table IV. Differences between groups A and B of perceived factors that facilitate coping with stress (Mann-Whitney U-test)

Factors	Rank Sum		U[c]	p[d]
	Group A[a]	Group B[b]		
A supportive atmosphere in the department	404782	82796	62896	0.0001
Absence of Covid-19 cases among staff	373675	79001	60086	0.0001
Recovering patients with COVID-19	410698	87804	67098	0.0001
Availability of gear and protective equipment	394530	88123	68023	0.002
Absence of COVID-19 infected among loved ones	407611	92890	72184	0.007
The likelihood to receive additional compensation for working with Covid-19 patients	301737	72808	56698	0.091
Relieving stress (relaxation, going info sports and so on)	375493	73386	55050	0.0001
Communication with family and friends to relieve stress and get support	400898	82739	62839	0.0001
Avoidance of COVID-19 media reports in particular on death connected cases	344816	78845	59342	0.0001
Ability to letting out one's emotions	296272	80875	62730	0.552
Getting information about the spread of Covid-19 only from legitimate sources	356996	80585	61279	0.0001

Notes: [a] – group respondents experiencing anxiety/fear, [b] – group respondents who is not experiencing anxiety/fear, [c] – Mann–Whitney U statistic, [d] – p-value of significance.

on a daily basis", "lack of staff in health care institutions", "difficulty getting to work" (p = 0.0001). Another large group of factors can be classified as those associated with physical and emotional exhaustion while providing care in special conditions. They comprised "irritability due to increased workload", "conflict between duty and personal safety", "physical exhaustion or fatigue", "complex challenge associated with treatment of Covid-19", "seeing patients with Covid-19 dying" and "observing colleagues being stressed" (p = 0.0001). The following factors associated with anxiety/ fear in connection with COVID-19 can be defined as informative: "absence of information about the exact time the outbreak will be under control" and "constant news about new COVID-19 cases in television/social networks" (p = 0.0001) (Table II).

Other factors that were strongly associated with anxiety/ fear related to COVID-19 could be potentially combined according to protective emotional reaction of the healthcare workers in attempts to avoid doing their professional duties using different pretexts. Such factors included "intentions to reduce contact with patients", "thoughts about quitting the job" and "thoughts about getting a sick leave" (p=0.0001). This group also included the following factors: "thoughts about quitting a job if one of colleagues gets COVID-19" and "thoughts about quitting a job if one of colleagues dies from COVID-19". One more group of factors that demonstrated a significant influence on anxiety/ fear related to COVID-19 was connected with discrimination of subjects who provided care to the patients with COVID-19. Such factors concerned "the feeling that employees who did not work with COVID-19 were avoiding you" and "the feeling that others were avoiding you because you might have had contact with coronavirus" (Table III).

The next group of factors is factors that facilitate coping with stress. These included "supportive atmosphere in the department" (p = 0.0001), "absence of Covid-19 cases among staff" (p = 0.0001), "recovering patients with Covid-19" (p = 0.0001), "availability of gear and protective equipment" (p = 0.002) and "absence of COVID-19 infected among loved ones" (p = 0.007), "relieving stress (relaxation, going info sports and so on)" (p<0.0001), "communication with family and friends to relieve stress and get support" (p<0.0001), "avoidance of COVID-19 media reports in particular on deaths connected cases" (p<0.0001), "getting information about the spread of Covid-19 only from legitimate sources" (p<0,0001). At the same time, the following factors such as "the likelihood to receive additional compensation for working with Covid-19 patients" (p = 0.091) and ability to letting out one's emotions (p=0.552) did not influence the ability of respondents to cope with stress (Table IV).

DISCUSSION

Most of the respondents had anxiety/fear related to COVID-19, that was associated with age. Among the factors that significantly influenced the feeling of anxiety/ fear related to COVID -19 in healthcare workers were stress due to the risk of COVID infection constant news about new cases of COVID-19, lack of staff in health care institutions, risk of transmitting COVID-19 to family or friends, perception of the risk of death from COVID-19 as high, perception of the threat of the COVID-19 pandemic spread as serious one. Results of our study partially coincide with results obtained by Tengilimoğlu D. et al. [17] who mentioned long working hours and lack of protective

equipment as the most important problems of the healthcare employees in relation with their work environment, healthcare workers feared to catch the virus and feared to contaminate COVID-19 virus to their family/parents. In the research, conducted in Tongji Hospital in China, less than 60% of participants had moderate or severe stress. From the higher five items that were moderate or severe stressors, fear of being infected, worrying about transmitting COVID-19 to their relatives and friends were mentioned [18], which corresponds with our research. Moreover, in our study, participants in group A had a median of 3 (which is the most severe score on this item), related to these stressors. A study by Elbay R.Y. et al. showed that female participants of the younger age had a higher level of stress [19]. However, our study found no significant gender-based differences, despite age. Also, a study conducted by Elbay R.Y. et al. revealed that those living with their spouse and children had lower scale scores related to stress, groups in our study did not differ significantly by the form of living.

CONCLUSIONS

The COVID-19 pandemic has had a significant impact on the psycho-emotional state of medical workers in Ukraine. The study found that the following factors cause a high level of anxiety / fear in them: the risk of contracting COVID-19, constant news of new cases of the disease, insufficient staff in medical facilities, the risk of transmitting COVID-19 to family or friends, a high risk of death from COVID-19, the threat of the spread of the COVID-19 pandemic. The data obtained in this study indicate an urgent need for the development and implementation of preventive and rehabilitation measures aimed at stabilizing the psycho-emotional state of health workers and improving the quality of care in the special conditions of the COVID-19 pandemic. Achieving these goals will be facilitated by taking into account the specifics of the response to COVID-19 and measures to reduce the effects of stress while enhancing the effects of stressors among health care workers.

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ORIGINAL ARTICLE

DUAL TRIGGER STRATEGY AFTER CONTROLLED STIMULATION INTRAUTERINE INSEMINATION CYCLE DID NOT INFLUENCE THE PREGNANCY OUTCOME COMPARED WITH STANDARD HCG TRIGGER ONLY PROTOCOL

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ABSTRACT

The aim: A prospective randomized comparative study was conducted to investigate the effect of dual trigger, using gonadotropin releasing hormone agonist with human chorionic gonadotropin (hCG) versus hCG alone, for ovulation trigger in controlled ovarian stimulation intrauterine insemination (IUI) cycle on pregnancy outcome.

Materials and methods: Ninety women were randomly allocated into equal groups to trigger ovulation for IUI cycle using either method; IUI was performed following 36-42 hours post triggering. Luteinizing hormone (LH) and progesterone levels were measured at insemination day.

Results: The baseline of demographic and clinical characteristics of both groups was similar. Progesterone level was higher in dual trigger group than in hCG alone group (1.61 versus 0.71 ng/mL, P≤0.0001); while LH level was lower in dual trigger group (19.35 versus 24.51 IU/L, P≤0.014). Furthermore, LH level at the day of IUI was higher in pregnant women than in non-pregnant (27.9 versus 20 IU/L, P≤0.007).

Conclusions: Pregnancy rate was equivalent in both groups. More intensive investigation is required to study the efficacy of the dual trigger in IUI cycle.

KEY WORDS: dual trigger, human chorionic gonadotropin, GnRH agonist, intrauterine insemination, pregnancy rate

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INTRODUCTION

Intrauterine insemination (IUI) is deemed to be the first procedure to choose among assisted reproductive techniques for infertility treatment due to its simplicity, relatively affordable ease of management, being low invasive and less expensive. Additionally, it's patient friendly compared to other *in vitro* fertilization techniques; with pregnancy rate ranging from 8.5 % to 21.4 % [1–3]. Ovarian stimulation induction prior to IUI is commonly used to treat infertile couples and may give better results [4-5] than natural cycle [6-7]. In natural ovulatory cycles, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) display a mid-cycle surge. This LH pulse surge initiates oocytes maturity and triggers ovulation. Using exogenous gonadotropin for controlled ovarian stimulation cycle was found to increase the pregnancy rate markedly [8]. Ovulation triggering with hCG is widely used in the clinical practice as a mid-cycle LH surrogate before insemination with two major functions. Firstly, it induces oocytes maturation that entirely depend on the LH component and activity of hCG and secondly, it serves as luteal phase recues owing to its extended half-life [9]. Improvement of the oocytes maturation and quality is significantly shown to affect the derived embryo developmental competence and implantation potential. The hCG has an advantage in replicating LH surge exceeding the natural cycle by inducing nuclear and cytoplasmic maturation via activation of the LH receptors on oocytes-cumulus complex, which finally affects resumption of meiosis through signaling pathway ended by activation of the maturation promoting factor [10]. HCG plays a major role in the maintenance of corpus luteum through its prolonged half-life and high progesterone secretion from the luteinized granulose cells, thus, supporting the implantation process. It may be also facilitating the trophoblastic invasion due to peri-trophoblastic immune tolerance development modification leading to blastocysts development in the endometrium [11]. Although the use of GnRH agonist (GnRHa) in IVF has been well established, its using in IUI is minimal. GnRHa can be used to induce (trigger) ovulation, either alone or in combination with hCG; both in natural or stimulated IUI cycles (as a physiological trigger causing both LH and FSH flaring from the pituitary). The shorter LH surge leading to oocytes maturation and ovulation is safe in polycystic ovarian syndrome (PCOS) patients. The additional FSH peak induces resumption of meiosis, to induce further oocytes maturation and higher number of mature oocytes than is expected with hCG trigger and corpus luteum formation through induction of LH receptors in the granulose [11]. Recently, physicians and fertility centers worldwide

have started to dismiss IUI and offer IVF due to the wide variability of indications and low overall pregnancy rates compared to IVF [12]. In attempt to retain IUI as a first choice of infertility treatment option for its simplicity and affordability, especially in developing countries, this study was designed.

THE AIM

To investigate, if dual trigger strategy can improve the pregnancy rate compared to traditional (only hCG) triggering protocol in controlled ovarian stimulated IUI cycle.

MATERIALS AND METHODS

A prospective randomized comparative study was conducted in the infertility clinic at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology Baghdad, Iraq, in period between October 2020 and April 2021. The study was approved by the Ethics Committee of institute and written consents were obtained from all couples participated in the study. A total of 90 couples (women, n=90) were enrolled in this study. All women included in the study were subjected to complete history and physical examination, gynecological examination, and an infertility checkup including basal hormonal analysis, tubal patency evaluation (by hysterosalpingogram, HSG or saline infusion sonography, SIS), transvaginal ultrasound (TVUS) for assessment of the uterine cavity. A seminal fluid analyses were also conducted in male partners. Inclusion criteria were the following: female age between 18-40 years, with at least one patent tube (diagnosed by either HSG or Hysteron contrast sonography) and normal uterine cavity. Women with primary or secondary infertility were included. Infertility due to sever male factors issues were excluded. Ovarian stimulation for all included women was started on cycle day three (CD3) and continued for five days (CD3-CD5) using 2.5 mg Letrozole (Femara®, Sanofi-Aventis France) two times daily, and 75 IU of Human Menopausal Gonadotropin (Menogon®, Ferring Pharmaceutical Switzerland) added according to ovarian response. Ovarian cycle was monitored (using follicular numbers, size, endometrial thickness and pattern parameters) by a 2D Trans vaginal ultrasound probe (Honda electronics; Japan). Examination was performed starting CD8, then every two to three days, till the day of ovulation trigger, when the follicles reached the average diameter between 18 to 24mm. At ovulation triggering time, women were divided randomly and equally into two groups. Group 1 was subjected to hCG standard protocol using hCG 250 mcg (Ovitrelle®; Organon USA), while women in group 2 received dual trigger (0.1mg Triptorelin; Ferring Pharmaceutical, Switzerland combined with 250 mcg Ovitrelle®; Organon USA) at ovulation trigger day. The TVUS scan was performed to monitor the ovulation. Furthermore, serum LH and progesterone (P4) levels were measured on the day of IUI. For this purpose, blood samples (5mL) were taken, using disposable syringe into clean gel plastic tube and left until clotted at 37 °C for 30 minutes, then centrifuged at 1000 rpm for 10 minutes. Serum samples were stored at -20 °C until the day of analysis.

The hormone assay was performed by MINI VIDAS® device according to the company instruction. Semen specimens were collected in a clean container and processed by swim-up procedure (1mL of liquefied semen samples were put beneath 1mL of pre warmed fertility cult culture medium then incubated for 45 minutes to allow superior spermatozoa to migrate from seminal plasma into culture medium). A single IUI was performed after 36-42 hours from trigger time in both groups, with 0.5-1mL of prepared semen aspirated from the top layer by IUI catheter and immediately inseminated in the prepared female for IUI. Daily treatment with progesterone vaginal suppositories (Cyclogest® 400mg; actvis) once a day was started from the day after IUI and continued for 14 days to induce luteal phase support. Pregnancy was diagnosed by B-hCG value of 25 IU/mL or above, done 2 weeks post IUI and clinical pregnancy diagnosed by TVUS for gestational sac was done 4 weeks later. Only pregnancies with a confirmed gestational sac were reported in the present analysis. Quantitative variables were presented in terms of mean ± SD, compared using an independent samples t-test; while chi-square test was employed to investigate the relationship between them. Significant level was set to $p < 0.05$ using the IBM SPSS Statistics for Windows, Version 23 to analyze the data.

RESULTS

The demographic characteristics of all women included in this study are presented in Table I, with no differences were identified between the study groups. However, the majority of women with secondary infertility were below 35 years old (the duration of infertility <5 years). Unexplained infertility was the most common cause of admission.

The clinical characteristics of both groups showed no differences; although most participants in each group underwent first trial of IUI (dual trigger group; 68.9% and hCG trigger group; 71.7%), Table II. The ovulation signs were obvious in most women in dual trigger group (80%) and hCG trigger group (84.4%), Table III. All women with no ovulation signs failed to get pregnant; while 29.7% of those who had signs of ovulation got pregnant with a significant association ($P \leq 0.012$) between ruptured follicle and pregnancy rate as presented in table IV.

The mean P4 levels were significantly higher in dual trigger group than in the standard hCG trigger group (1.61 versus 0.71 ng/mL, $P \leq 0.0001$); while the mean levels of LH were significantly lower in dual trigger (19.35 versus 24.51 IU/L, $P \leq 0.014$) as shown in table V. The mean LH value at day of IUI was significantly higher in women who got pregnant versus not pregnant women (27.9 versus 20 IU/L, $P \leq 0.007$), table VI. No significant differences between the two trigger strategies concerning pregnancy rate were recorded, table VII.

Table I. Clinical characteristics of all women participated in this study according to the ovulation trigger strategy used.

		Dual trigger group		hCG only group		p-value
		No.	%	No.	%	
Age (years)#	<25years	9	20.0	11	24.4	0.694
	25---34	28	62.2	24	53.3	
	≥35years	8	17.8	10	22.2	
	Mean±SD (Range)	29.42±5.40 (18-40)		29.04±6.18 (17-40)		0.758
BMI (Kg/m2)#	Normal (18.5-24.9)	19	42.2	24	53.3	0.573
	Overweight (25-29.9)	15	33.3	12	26.7	
	Obese (≥30)	11	24.4	9	20.0	
	Mean±SD (Range)	25.92±5.03 (19-37)		24.22±4.44 (18-33.9)		0.092
Type of Infertility*	Primary	22	48.9	18	40.0	0.396
	Secondary	23	51.1	27	60.0	
Infertility Duration# (years)	<5years	37	82.2	34	75.6	0.449
	5---9	4	8.9	8	17.8	
	≥10years	4	8.9	3	6.7	
	Mean±SD (Range)	3.66±3.44 (1-16)		4.02±2.74 (1-13)		0.578

* Pearson Chi-square test (χ2-test), significant difference between percentages categorical variables # two independent t-tests used to compare mean for continuous variables
Note. SD: standard deviation; BMI: body mass index; №: number

Table II. The clinical and stimulation characteristic in the women in both study groups

		Dual trigger group		hCG only group		p-value
		No.	%	No.	%	
Cause of infertility	PCOS	10	22.2	13	28.9	0.892
	UEI	25	55.6	22	48.9	
	Male	6	13.3	6	13.3	
	Endometriosis	1	2.2	2	4.4	
	Combined	3	6.7	2	4.4	
IUI Trial	First	31	68.9	32	71.1	0.214
	Second	12	26.7	8	17.8	
	Third	1	2.2	5	11.1	
	Fourth	1	2.2	-	-	
Gonado Ampule	One	16	35.6	13	28.9	0.909
	Two	15	33.3	16	35.6	
	Three	9	20.0	11	24.4	
	Four	5	11.1	5	11.1	
Dominant follicle (DF) No	one	24	53.3	24	53.3	0.393
	Two	17	37.8	13	28.9	
	Three	4	8.9	8	17.8	

Note. Pearson Chi-square test (χ2-test); №: number

DISCUSSION

The demographic characteristics of both groups had shown no significant difference, including body mass index (BMI), length of infertility, type of infertility; both share the same inclusion and exclusion criteria. The ovulation signs in both groups did not alter due to the used ovulation triggering protocol, which is comparable with the finding of [13], who performed a retrospective study

Table III. The signs of ovulation in both groups

After 36 -42 hours		Dual trigger group		hCG only group		p-value
		No.	%	No.	%	
Ovulation	Yes	36	80.0	38	84.4	0.581
	No	9	20.0	7	15.6	
Pearson Chi-square test (χ2-test)						

Note. Pearson Chi-square test (χ2-test); №: number

Table IV. The association between pregnancy outcome and ovulation

Ovulation	IUI cycle outcome		Total (%)n= 90	p-value
	Pregnant (%)n= 22	Not pregnant (%)n= 68		
Yes	22 (29.7)	52 (70.3)	74 (82.2)	0.012
No	0 (0)	16 (100.0)	16 (17.8)	

Note. Pearson Chi-square test (χ2-test); n: number of cases

Table V. The progesterone and LH at the day of IUI in both groups

		Dual trigger group		hCG only group		p-value
		No.	%	No.	%	
Progesterone (ng/mL)	<.0.5ng/mL	8	17.8%	12	26.7%	0.0001*
	0.5---	8	17.8%	23	51.1%	
	1.0---	15	33.3%	9	20.0%	
	1.5---	3	6.7%	1	2.2%	
	≥2.0ng/mL	11	24.4%	-	-	
	Mean±SD (Range)	1.62±1.48 (0.13-6.79)		0.71±0.29 (0.30-1.5)		0.0001#
LH (IU/L) IUI	<20 IU/L	33	73.3%	20	44.4%	0.043*
	20---	8	17.8%	14	31.1%	
	30---	3	6.7%	9	20.0%	
	≥40 IU/L	1	2.2%	2	4.4%	
	Mean±SD (Range)	19.35±8.64 (3.0-57.9)		24.51±10.76 (7.8-70.3)		0.014#

Note. *Pearson Chi-square test (χ2-test), the significant difference between percentages set at 0.05 levels; #Independent t-test, the significant difference between means set at 0.05 levels; №: number

comparing three types of ovulation triggering strategy in IUI cycles (hCG trigger, GnRHa trigger, and dual trigger). Le et al., (2019) comparing hCG and GnRHa triggers, showed no differences in ovulation rate. Dominant follicles characteristics, whether ruptured or not, were examined by ultrasonography at the day of the IUI. The results of this study indicated an association between the ovulation signs and pregnancy rate; women whose follicles failed to rupture (100%) were unable to conceive, whereas 29.7% of those who had ruptured follicles had conceived (P≤ 0.012). Whereas no differences in dominant follicle ruptures, and pregnancy rates 90% of IUI cycles were noticed between the hCG and dual trigger groups [15]. Moreover, positively correlation was noticed between the occurrence of follicle rupture and clinical pregnancy rate in IUI cycle [15-16]. Furthermore, [17-18] indicated that, IUI cycle outcomes are independent to the ovulation status documented before insemination. Accordingly, no differences were noticed in clinical pregnancy rate between ruptured and non-ruptured follicles; whether follicle was ruptured before or after insemination it did not affect the pregnancy rate in IUI cycle. The serum progesterone levels did not differ between pregnant and non-pregnant women in this study; the progesterone elevation had no effect on cycle outcome, which were found in similar studies by [19-20].

The correlation of progesterone level and pregnancy outcome in ART is still debatable, as higher progesterone levels may be related to exogenous FSH. Ovarian stimulation methods induce greater levels of steroidogenic activity, although the mechanism is different in IUI, as there is a minimal ovarian stimulation. Alternatively, it might be explained by increased LH production or changes in the LH receptor sensitivity [21-23]. It has been shown that progesterone levels on trigger day or on day of IUI

Table VI. Comparison of the hormonal levels at the day of IUI in respect to the cycle outcome

Hormonal parameter at day of IUI	IUI cycle outcome		p- value
	Pregnant Mean ± SD	Not pregnant Mean ± SD	
LH (IU/L)	27.9 ± 11.7	20.0 ± 8.7	0.007
Progesterone (ng/mL)	1.04 ± 0.86	1.2 ± 1.2	0.516

Table VII. Display of the pregnancy rate in each group

		Dual trigger group		hCG only group		P value
		No.	%	No.	%	
Pregnancy	Pregnant	10	22.2	12	26.7	0.624
	Not pregnant	35	77.8	33	73.3	

Note. *Pearson Chi-square test (χ2-test); №: number

are not significant predictors of cycle outcome, and progesterone elevation has no significant impact on cycle outcome. However, progesterone levels between 1.0 and 1.25ng/mL may lead to good clinical pregnancy outcomes [24]. Other research revealed that progesterone threshold (1.5ng/mL) with the maximum pregnancy rate was found in the range of 0.5-1.5 ng/dl, and the lowest rate found when progesterone level was higher than 1.5ng/dl [25].

According to another meta-analysis research by [28], the raising of progesterone was shown to be associated with lower fertilization and pregnancy rate due to negative effects on endometrium receptivity and synchronization between the endometrium and embryo. The clinical pregnancy rates and implantation rates were lower in women with low LH levels at any time throughout the cycle. This unfavorable outcome might be related to poor oocytes quality or a detrimental influence affecting the endometrium; however, the mechanism is unclear and requires additional research. According to some research, serum LH less than 15 mIU/mL a day after GnRH-a administration indicates an inadequate hormone response to the trigger, resulting in considerably lower oocytes maturity compared to those with appropriate response (post-trigger LH > 15 mIU/mL). According to the same study above, LH levels less than 0.5 mIU/mL before the hCG trigger day are associated with impaired endometrial receptivity; so, adding low-dose of hCG to raise LH activity before ovulation induction appears to cause a significant reduce of endometrial stromal cell apoptosis, implantation improvement and better reproductive outcome. In the current study, there was no significant difference in pregnancy outcomes between the hCG trigger and dual trigger groups. These findings are consistent with studies by [15-18]. Contradicting, [29] found that the dual trigger group had a higher clinical pregnancy rate than classical hCG triggering group (50.7% vs. 40.1%).

CONCLUSIONS

Dual trigger protocol could be more effective than conventional hCG triggering procedure with comparable pregnancy rate. More investigations in bigger groups are required to improve the effectiveness of the dual trigger protocol. Studying the efficacy of the dual trigger strategy in women with PCOS family history is recommended.

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All experimental Protocols were approved under the Al-Nahrain University and all experiments were carried out in accordance with approved guidelines.

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ORIGINAL ARTICLE

THE ROLE OF LEPTINRESISTANCE IN THE PATHOGENESIS OF OBESITY IN PREGNANT WOMEN

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ABSTRACT

The aim: To investigate the relationship between leptin resistance, lipid and carbohydrate metabolism, blood pressure in obese pregnant women.

Materials and methods: Under observation were 65 women (main group) with obesity (I degree -27 women, II degree - 24 women, III degree - 14 women) in the II trimester of pregnancy, who were hospitalized in the Department of Pathology of Pregnancy KNP «Maternity Clinical house №1 "in Lviv during 2017-2020 on preeclampsia of varying severity, which were sent for inpatient treatment by women's clinics. The control group consisted of 30 healthy pregnant women without obesity.

Results: Serum leptin in obese women was directly correlated with BMI ($r = 0.66$, $p<0.001$), body weight ($r = 0.29$, $p<0.05$), total cholesterol (cholesterol) ($r = 0, 37$, $p<0,009$), low density lipoproteins (LDL cholesterol) ($r = 0.33$, $p<0.05$) and inversely with high density particles (HDL cholesterol) ($r = -0.37$, $p<0.02$). Studies of carbohydrate metabolism indicate the following correlation coefficients of BMI with glucose level $r = 0.351$; $p<0,001$, BMI with the level of C-peptide $r = 0,450$; $p<0,001$, BMI with HOMA index $r = 0,1504$; $p = 0.036$. Inverse correlations of C-peptide were detected with the level of P ($r = -0.169$; $p = 0.025$).

Conclusions: The discovery of the relationship between leptin resistance, lipid and carbohydrate metabolism, blood pressure indicates the possibility of using signs of leptin resistance to prevent complications during pregnancy in the second trimester.

KEY WORDS: leptin, cholesterol, glucose, C-peptide, HOMA index

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INTRODUCTION

The problem of obesity is becoming increasingly important today and poses an economic, medical and social danger to human life, regardless of social or occupational distribution, place of residence, age or gender. Obesity is a chronic disease that manifests itself in pathological deposition of fat in the body and excessive weight gain. In different countries, about 10% of the health budget is spent on the treatment of obesity and related pathologies. Only 50% of the population of Ukraine has a normal weight. Instead, more than 22% are obese. According to statistics, the mortality rate of young people due to overweight is 12 times higher than for people with normal body weight.

Compared with 1960, the caloric content of food increased by 24-26%, probably due to increased consumption of refined carbohydrates, transgenic fats, sodium, potassium, fiber, non-compliance with water regime, physical activity. An important role in this is played by increased levels of stress hormones, mutations in signal receptors to insulin and leptin molecules, which are one of the causes of obesity. Other factors are hedonistic consumption of food, which brings pleasure, reduces stress levels, as well as genetic predisposition to obesity, which (according to the literature) accounts for 40-70% of all causes, sedentary lifestyle, smoking, psychosocial factors.

Table I. Characteristics of lipid metabolism of examined pregnant women, M+m (95% CI)

Indicator	Clinical group		p between groups by t-test
	Main group (n=65)	Control group (n=30)	
Cholesterol, mmol/l	5,58±0,10	5,15±0,17	0,014
Triglycerides, mmol/l	1,87±0,07	1,76±0,12	0,812
LDL, mmol/l	2,68±0,05	2,34±0,11	0,30
VLDL, ммоль/л	0, 468±0,21	0, 412±0,20	0,23
HDL, mmol/l	1,58±0,02	1,69±0,02	0,609
Atherogenicity index (AI)	2,43±0,06	2,07±0,10	0,004

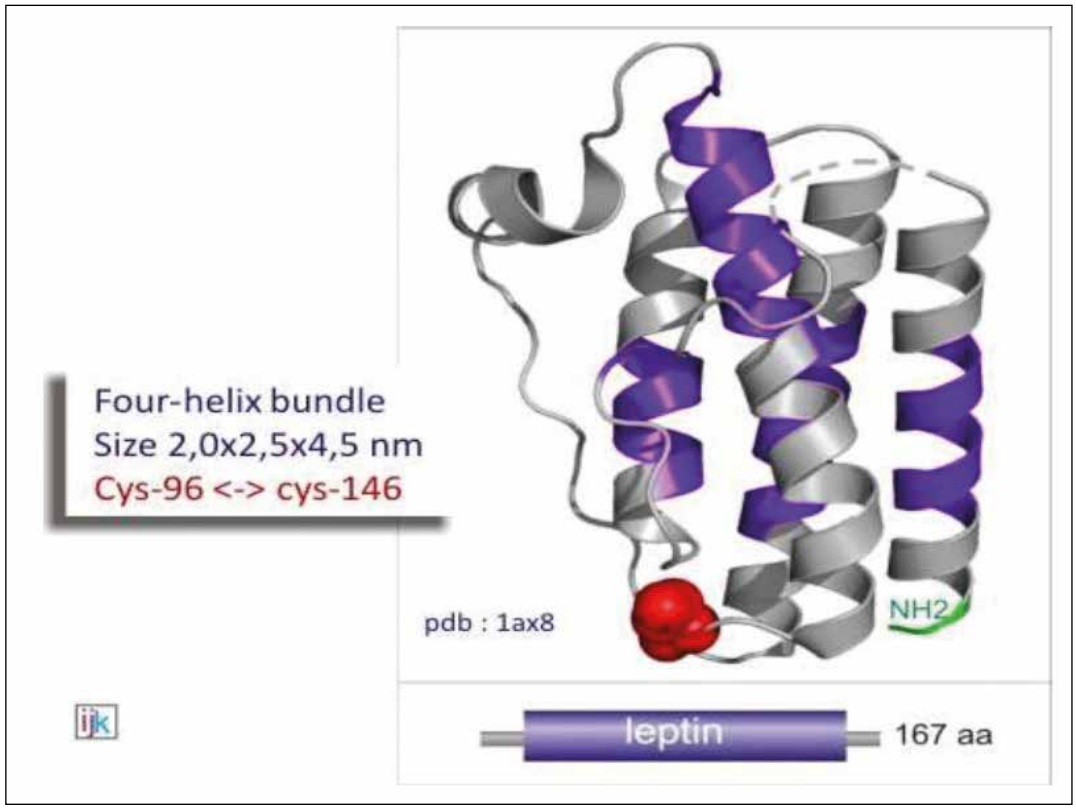

Fig.1. The structure of the leptin molecule.

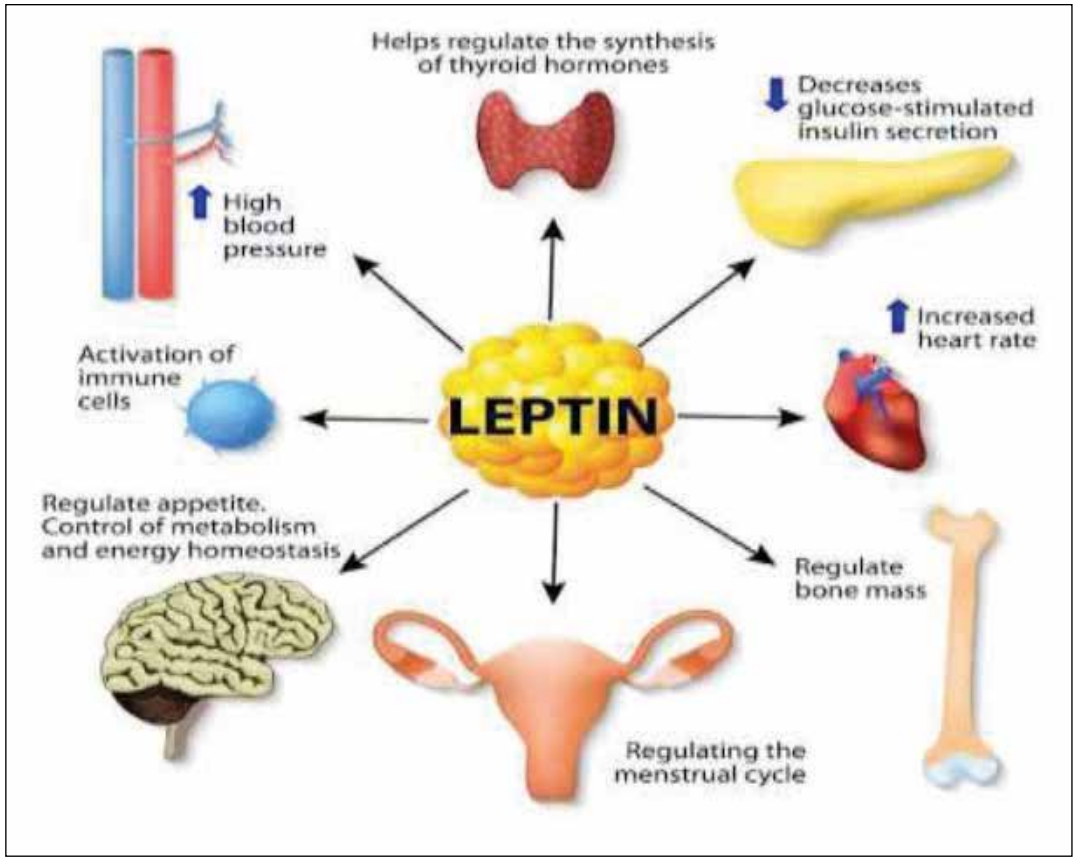

Fig.2. Main effects of leptin

Today, the theory of economical genotype remains relevant, the basis of which is hyperinsulinemia in conditions of increased food intake and, as a result, the emergence of excess body weight.

Recently, the role of adipose tissue, which is considered an endocrine and paracrine organ that produces a number of hormonally active substances - adipokines (adipose derived hormones), which cause disorders of carbohydrate

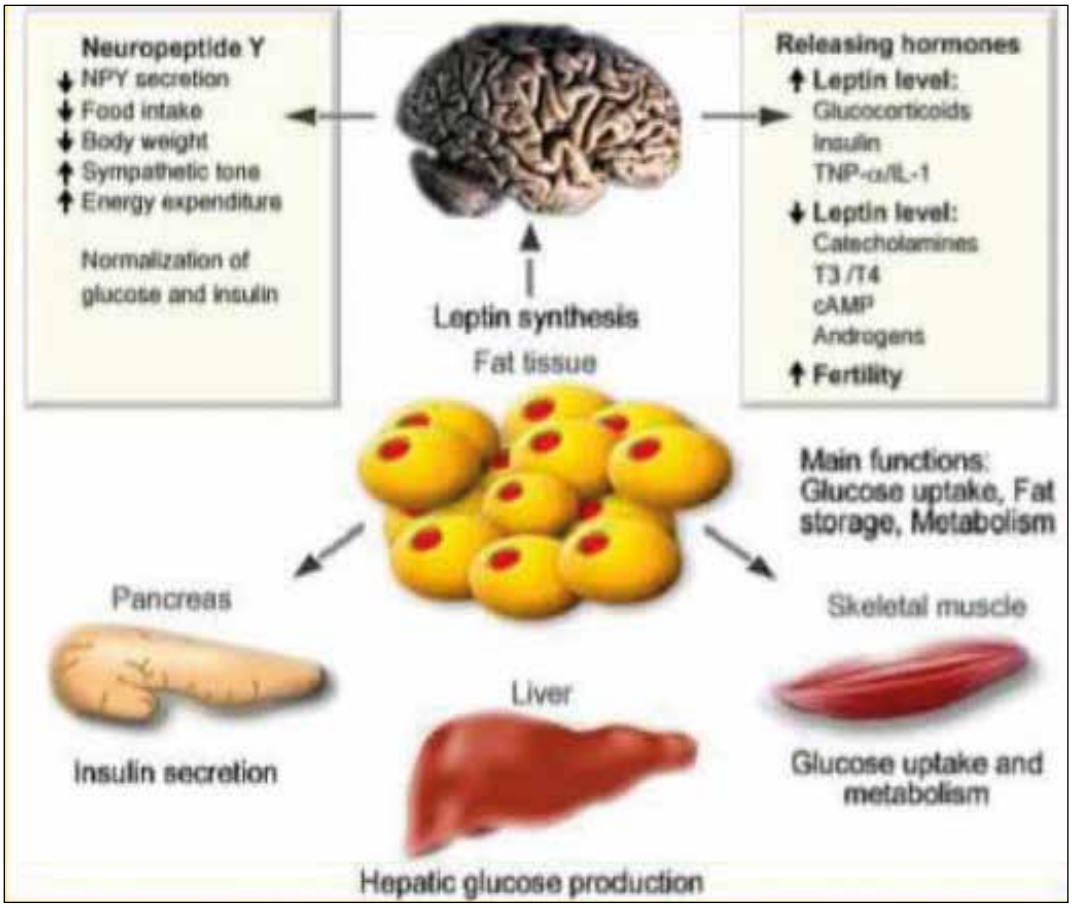

Fig. 3. Scheme of pathophysiology of lipid metabolism.

Table II. Characteristics of carbohydrate metabolism in women of the examined groups, M±m (95% CI)

Indicator	Clinical group		p between groups by t-test
	Main group (n=65)	Control group (n=30)	
Insulin, μOd / ml	10,54±0,35	10,86±0,41	0,05
Blood glucose, mmol/l	4,58±0,07	3,93±0,07	<0,001
C-peptide ng/ml	1,65±0,05	1,03±0,04	<0,001
Glycosylated hemoglobin,%	4,80*	4,80*	0,448**
HOMA index	2,02±0,06	1,90±0,04	0,041

Note: *- Data are presented in the form of Me [25%; 75%]; **- by U-criterion

Table III. Leptin levels and blood pressure in examined pregnant women, M±m (95% DI)

Indicator	Clinical group				p between groups by t-test
	Main group (n=65)			Control group (n=30)	
	I degree	II degree	III degree		
Leptin ng / ml	31,16±0,04	39,86±0,05	48,59±0,03	17,12±0,03	<0,05
SBP mm Hg	143,0±1,2	152,3±1,4	163,4±4,3	135,8±1,1	<0,001
DBP mm Hg	80,3±1,8	86,5±2,3	90,4±2,1	85,6±2,1	<0,001

and lipid metabolism, regulate inflammation and immune homeostasis, has been significantly revised [1-8]. The most famous of these is leptin [8].

The hormone leptin is a low molecular weight α-helix protein (16 kDa, 167 amino acids), which by its crystal structure belongs to cytokines (Fig.1). Leptin molecules circulate in the blood in both free and protein-bound form.

Leptin plays an important role in the regulation of metabolism and functions of the neuroendocrine system, including the pituitary gland, hypothalamus, adrenal glands, immune system. (Fig.2).

Leptin, which is produced by adipocytes, interacts with the long isoform of the leptin receptor LRb in the nuclei of the hypothalamus, activating metabolism, sympathetic

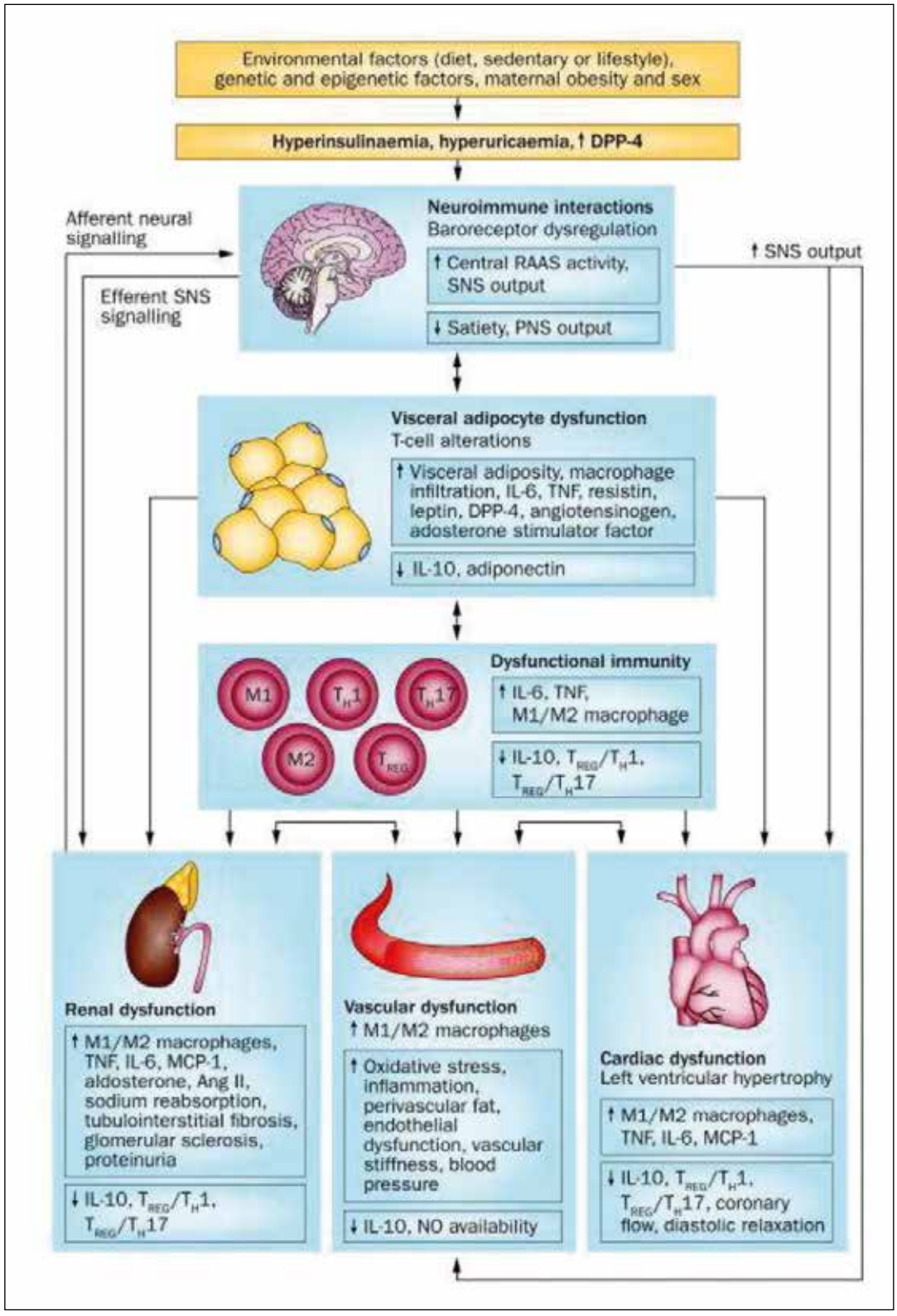

Fig.4. Pathogenesis of arterial hypertension in obesity.

nervous system tone, thyroid function, synthesis of sex hormones and growth hormones. Leptin enhances the immune response by activating LRb on T cells, and increases insulin sensitivity and regulates body weight by inhibiting satiety.

In recent years, there have been experimental data on the involvement of leptin in the induction and regulation of chronic inflammatory processes and degenerative diseases, including autoimmune pathologies, type 2 diabetes [1]. In humans, leptin is synthesized by cells of white and brown adipose tissue, skeletal muscle, stomach and placenta. Subcutaneous adipocytes produce 2.5 times more leptin than visceral fat. Leptin acts on the centers of hunger and satiety in the hypothalamus, is involved in the regulation of energy homeostasis and controls body weight by reduc-

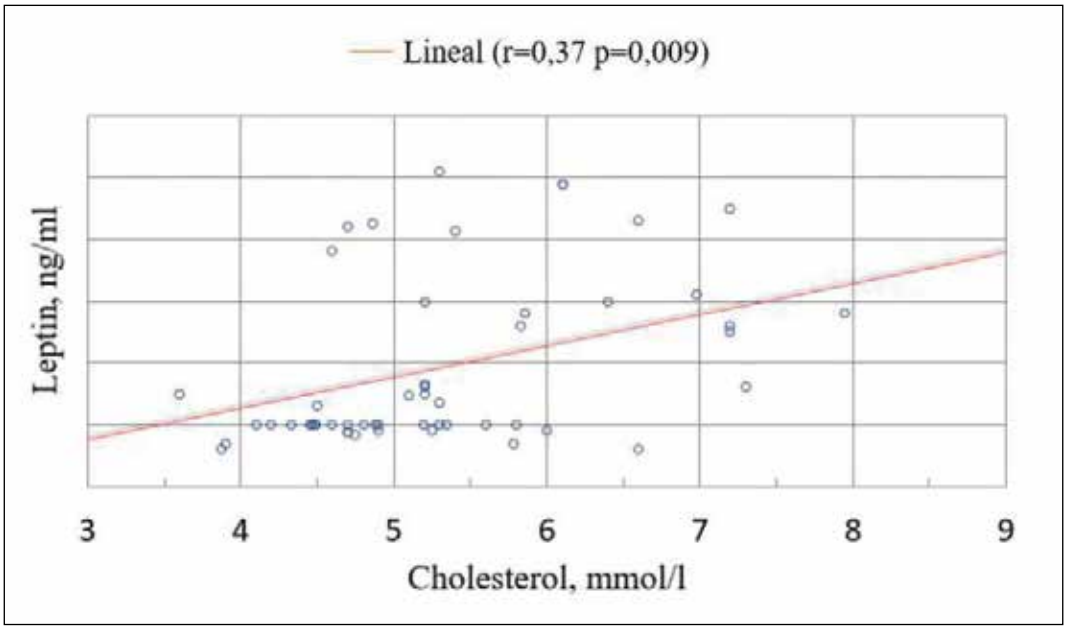

Fig.5. Dependence of leptin level on cholesterol concentration.

ing biosynthesis and the release of neuropeptide Y, which causes a feeling of hunger. Leptin secretion is not constant: its peak is observed in the afternoon, the minimum level after midnight. Obesity causes compensatory resistance of the hypothalamus to the central action of leptin, which subsequently leads to hyperleptinemia by the mechanism of negative feedback. It is believed that leptin is a link between adipocytes and β-cells of the pancreas and stimulates insulin secretion by reducing insulin sensitivity. There was a direct relationship between leptin levels and the degree of insulin resistance, taking into account changes in adipose tissue volume in postmenopausal women. In both in vitro and in vivo studies, leptin has been shown to function as a growth factor, stimulating angiogenesis, hematopoietic cell proliferation, and pancreatic β-cells. In addition, by suppressing the synthesis of neuropeptide Y in the hypothalamus, leptin stimulates the secretion of growth hormone in the pituitary gland. It is believed that leptin levels are a signal marker of sufficient accumulation of adipose tissue [2]. This process is necessary for puberty, regular menstrual cycles and provides the ability to reproduce. In healthy children in the prepubertal period, leptin levels increase in parallel with weight gain, reaching a maximum during puberty. Thus, leptin is considered as one of the triggering factors that initiate puberty [3,4].

Population studies in a number of European countries and the United States in the period 1990-2005 found an increase in the proportion of women of childbearing age with obesity from 9.9% to 16%. Adipose tissue is an endocrine organ that produces adipokines – leptin and adiponectin, which regulate fat metabolism and the risk of tumors, including breast cancer, endometrium [5,6]. As a rule, the level of leptin in the blood of women with a high (>25) body mass index is also elevated. This leads to impaired ovarian secretion of gonadotropic hormone and steroids, abnormal ovarian maturation, ovulation disorders and defects in endometrial development, as well as defects in implantation and early embryogenesis, which adversely affect female fertility. Normally, the concentration of free leptin in the serum reaches a maximum in the last luteal phase and decreases in the early follicular phase. In obesity, this cyclic content of leptin, as well as estradiol, is often disrupted and affects the ovulation process. In the luteal phase of the cycle, leptin stimulates apoptosis, as well as the synthesis of prostaglandin F2α, activating the regression of the corpus luteum. Leptin concentration balance provides luteolysis and apoptosis of corpus luteum cells in the absence of pregnancy. However, during fertilization and implantation of the egg, this balance is important for the normal development of the corpus luteum. In the culture of luteinized granulosa cells obtained from women participating in the IVF program, excess leptin concentration suppressed the expression of estradiol by these cells. It is noted that the violation of the normal balance of leptin adversely affects ovarian function, reducing the secretion of estradiol in granulosa cells. In addition, leptin can regulate ovulation by stimulating proteolysis and the release of follicle contents [7].

During pregnancy, the level of leptin in the blood rises, and after childbirth and abortion – falls. During pregnancy, leptin is additionally produced in the placenta and amniotic membranes, as well as in uterine tissues, inhibiting the contraction of uterine smooth muscle [3,8]. Recently, there are data on the pleiotropic effect of leptin on reproduction and pregnancy. In particular, the expression of leptin in the placenta enhances the proliferation and differentiation of trophoblast cells. Leptin production in the placenta is regulated by 17β-estradiol, which plays a key role in blastocyte implantation, in trophoblast differentiation and invasion, as well as in the regulation of uterine vascular growth and in the activation of protein kinase signaling pathways [9].

With obesity and abnormal serum leptin concentrations, there are usually pathological changes in the endometrium

and a low probability of implantation of a fertilized egg in the uterus, as well as the usual miscarriage [9,10]. Leptin receptors are found in the integumentary and glandular epithelium of the endometrium, their interaction with leptin activates the proliferation of stroma cells and endometrial epithelium, which ensures successful implantation of the egg. There are data on the contribution of leptin in the pathogenesis of endometriosis by autocrine and paracrine regulation [11, 12]. A multivariate analysis of 2527 married childless women and a control group of married women with many children shows that with increasing weight they increase the risk of cycles without ovulation, and the risk of low fertility is statistically significant at a body mass index > 23.9 [13]. As a rule, hyperleptinemia and leptin resistance are observed in obesity. This resistance is caused by several reasons: disruption of the production of various isoforms of the leptin receptor, inactivation of leptin molecules due to binding to blood proteins, disruption of hormone transport across the blood-brain barrier and insulin resistance. Hyperinsulinemia and insulin resistance have been shown to be associated with high blood leptin levels, regardless of body mass index [14].

Leptin regulates a number of neuroendocrine functions. In particular, disruption of the interaction of leptin molecules with its receptor leads to hypothalamic amenorrhea. Binding of leptin molecules to the long leptin receptor isoform mediates signal transduction and stimulates the synthesis of neuropeptides in the hypothalamus, which play a key role in puberty and reproduction. Mutations in the *Lep* gene encoding leptin in humans have been described, leading to hypolectinemia and delayed pituitary gonadotropic function with the development of hypogonadism due to gonadotropin-releasing hormone (GnRH) deficiency. Mutation of the *Lep* gene associated with congenital leptin deficiency, moderate obesity and signs of hypogonadotropic hypogonadism. A rare mutation in exon 6 of the LepR gene (PZ16T) is also associated with early obesity, severe hyperphagia, hypogonadotropic hypogonadism, neuroendocrine and metabolic dysfunction [15, 16,17].

Leptin is not synthesized by ovarian cells, but its concentration in follicular fluid is close to the concentration in the blood. The effect of leptin on ovarian function is ambiguous and depends on the stage of puberty. Leptin has been found to affect the activity of steroidogenic enzymes and folliculogenesis.Using immunocytochemical methods, transcripts of Lep and LepR genes (mRNAs) encoding leptin and its receptors were detected in the ovaries of adult women and adolescents, as well as in the embryonic ovaries at 2-3 weeks of gestation. The expression of leptin and the long isoform of its receptor, as well as the corresponding mRNA, were detected in oocytes of primordial and more mature follicles, and the expression of leptin in – granulosa cells [18]. There are also data on the role of leptin in the development of polycystic ovaries.It has been shown that in patients with this syndrome, who reduced body weight to normal values, the menstrual cycle normalized, oligo- and anovulation were observed much less frequently, and the probability of pregnancy increased. This is due to the restoration of the balance of hormones that regulate the development of follicles and egg maturation [5]. Hyperleptinemia, which is associated with overweight and obesity, is considered a risk factor for chronic oligo- or anovulation, which is caused by both insulin resistance and leptin-dependent ovarian dysfunction [19] (Fig.3).

There are data in the literature on the role and importance of leptin in hypertension (AH) [4, 9, 6, 10], but they are not yet final. Thus, the determination of leptin in 123 patients with hypertension showed that with increasing its level there is not only a progressive increase in body mass index and waist circumference, but also systolic and diastolic blood pressure (BP), insulin and insulin resistance index HOMA [5]. Some researchers consider leptin to be one of the means of regulating blood pressure. Evidence of this is that leptin receptors are found in the cells of the adrenal medulla, which may indicate the regulation of leptin synthesis of catecholamine vasopressors. At the same time, high levels of catecholamines reduce leptin secretion by activating beta-adrenergic receptors (Fig. 4).

Another mechanism for raising blood pressure under the action of leptin is the activation of the sympathetic nervous system under its influence, which leads not only to increased heat production and energy use [12], but also to increased blood pressure. This is confirmed by the fact of a significant correlation of leptin with the level of adrenoreactivity of the organism (r = 0.8) under conditions of increased body weight, accompanied by a change in the osmotic resistance of erythrocytes under the influence of beta-blockers [4]. Increased leptin levels were observed in the combination of hypertension and tachycardia [3], which can also be explained by the activation of the sympathetic nervous system. The level of leptin decreased with the treatment of hypertension, which, however, depended on the sex of the patient: in men the level of leptin decreased significantly, while in women the decrease in leptin was less pronounced. That is, patients with hypertension have a leptin-dependent mechanism of hypersympathicotonia, which changes under obesity and loses linear dependence, which is explained by the development of insulin resistance (IR) and increased secretion of angiotensin.

The pathogenesis of hypertension in metabolic syndrome (MS) involving leptin is proposed. It is based on IR and compensatory hyperinsulinemia, and further effects are realized through leptin, which correlates with body mass index, regulates hunger associated with activation of the sympathetic nervous system, endothelial dysfunction, vasoconstriction, increased total peripheral vascular resistance and cardiac resistance. with increasing sodium reabsorption and hypervolemia [11]. However, in the clinic there are often large fluctuations in the amount of leptin, which makes it difficult to interpret the data. Thus, in patients with hypertension under conditions of normal body weight, the level of leptin was 16.9 ± 15.3 ng / ml, under conditions of overweight - 15.1 ± 12.5 ng / ml, while under conditions of obesity - 56.4 ± 41.2 ng / ml [4], and significant fluctuations in the obtained levels attract attention. According to other data, the leptin levels of patients with

hypertension were 5.42 ± 0.21 ng / ml, 9.69 ± 0.25 ng / ml and 17.71 ± 1.33 ng / ml, respectively [5].

These debatable issues and uncertain levels of leptin in hypertension determine the feasibility of further research.

THE AIM

To study the relationship between leptin resistance, lipid and carbohydrate metabolism, blood pressure in pregnant women with obesity in the second trimester of pregnancy.

MATERIALS AND METHODS

Under observation were 65 women (main group) with obesity (I st. -27 women, II st. - 24 women, III st. - 14 women) in the II trimester of pregnancy, who were hospitalized in the Department of Pathology of Pregnancy KNP «Maternity Clinical House №1 "in Lviv during 2017-2020 on preeclampsia of varying severity, which were sent for inpatient treatment by women's clinics. The control group consisted of 30 healthy pregnant women without obesity. Patients were randomized by age and pathology.

Determination of leptin was performed by enzyme-linked immunosorbent assay (ELISA) on a TESAN spectrophotometer using the LDN Leptin ELISA test system (Germany).

The degree of obesity was determined by the body mass index (BMI) - height-weight ratio, which is determined by the formula: weight in kilograms (m) divided by height in m^2 (h).

$$BMI = \frac{m}{h^2}$$

The weight at BMI from >18.5 to <25 kg / m2 is considered normal. If the BMI is > 30-35 kg / m2– it is obesity of the 1st degree, with BMI > 35-40 kg / m2– obesity of the 2nd degree, with BMI > 40 kg / m2– of obesity of the 3rd degree. The results were calculated by the methods of parametric (under Gaussian distribution) and nonparametric statistics.

RESULTS

The study of the somatic history of pregnant women of the main group revealed: hypertension (19 women) 30%, moderate myopia of both eyes - (13 women) 20%, anemia of pregnant women - (6 women) 10%, varicose veins of the lower extremities - (12 women) 10%, chronic tonsillitis - (6 women) 10%.

Peculiarities of reproductive history and menstrual function of the main group of pregnant women were: primary infertility - (39 women) 60%, polycystic ovary syndrome - (26 women) 40%, cervical erosion - (26 women) 40%, miscarriages - (13 women) 20%, stillborn pregnancies - (13 women) 20%, late menarche (19 women) 30%, menstrual disorders - (13 women) 20%.

Among the complications of pregnancy in women of the main group prevailed: early preeclampsia - (58 women) 90%, the threat of premature birth - (52 women) 80%, SARS - (26 women) 40%, premature discharge of amniotic fluid - (26 women) 40 %, disorders of fetal-placental blood flow - (32 women) 50%, scar on the uterus - (26 women) 40%, DFD - (26 women) 40%, premature birth - (6 women) 10%, cervical suture - (12 women) 20%.

In women of the control group somatic anamnesis was complicated by diffuse goiter - (9 women) 30%, anemia in pregnant women (3 women) 10%, varicose veins in (3 women) 10%, chronic tonsillitis in (3 women) 10%.

Among the features of reproductive history and menstrual function were primary infertility in (3 women) 10%, chronic salpingo-oophoritis in (6 women) 20%, polypectomy in (2 women) 6%, miscarriages in (1 woman) 3%, adenomyosis in (2 women) 6%, menstrual disorders in (3 women) 10%.

The course of pregnancy in women of the control group was complicated by acute respiratory viral infection (ARI) in (9 women) 30%, early preeclampsia in (6 women) 20%, the threat of premature birth (in 6 women) 20%, anemia in pregnant women (3 women) 10 %, impaired fetal-placental blood flow in (3 women) 10%.

When comparing the level of leptin in the serum of pregnant women of the control group and pregnant women of the main group with obesity of the first degree, the level of leptin in the control group was 17.12 ng / ml, in pregnant women with obesity of the first degree. - 31.16 ng / ml, which is 1.82 times more than in pregnant women without obesity ($p<0.05$).

When examining pregnant women with obesity II degree leptin levels were 2.14 times higher than in pregnant women of the control group, and in pregnant women with obesity III degree - 2.6 times, respectively ($p<0.05$).

It is noteworthy to compare the absolute value of leptin levels in pregnant women with obesity of varying degrees. Thus, in obesity of the second century. the level of leptin is higher by 8.7 ng / ml (1.3 times) in comparison with pregnant women with obesity of the first degree. With obesity of the III degree in comparison with obesity of the I degree leptin level at 17.43 ng / ml (1, 28 times), and in comparison with obesity II degree – higher by 7.8 ng / ml (1, 13 times) ($p<0.05$) (Table III).

Excessive weakness in the main group of pregnant women can be explained by the presence of digestive disorders and impaired bile secretion. Slight pain or a feeling of distension in the right hypochondrium or epigastric region was noted by (46 women) 71% of pregnant women, dyspeptic symptoms – (52 women) 80%. Ultrasound in (23 women) 15% of pregnant women showed an increase in liver size, (20 women) 31% had indications of chronic cholecystitis. A similar trend was not observed in pregnant women of the control group: liver enlargement was not detected in any case, signs of chronic cholecystitis occurred in (1 woman) 3%. That is why it was considered necessary to investigate the indicators of lipid and carbohydrate metabolism in women of prospective groups (table I). The results of the study of the lipid profile, shown in table I, indicate the presence of significant interdependence (from $p<0.05$ to $p<0.01$) of high levels of cholesterol, LDL, VLDL and atherogenic index from BMI of pregnant women.

According to Pearson's correlation analysis, blood leptin was directly correlated with BMI (r = 0.66, p<0.001), body weight (r = 0.29, p<0.05), total cholesterol (cholesterol) (r = 0). , 37, p<0,009), low-density lipoproteins (LDL cholesterol) (r = 0.33, p<0.05) and vice versa with high-density particles (HDL cholesterol) (r = -0.37, p<0, 02) (Fig. V).

Given the affiliation of the main group of women to the risk group for carbohydrate metabolism disorders during gestation, an oral glucose tolerance test was performed at the time of registration of the pregnant woman. His results showed that the level of glucose in women of the main group exceeded the corresponding values in pregnant women of the control group by 12.8% (p<0.001) in the study on an empty stomach and 10.8% after glucose loading (p = 0.004).

The results of the study of carbohydrate metabolism, shown in table II, indicate a significant interdependence (from p<0.05 to p<0.001) levels of blood glucose, C-peptide and HOMA-IR index from BMI of pregnant women.

The corresponding correlation coefficients were equal to: BMI with glucose level – r = 0.351; p<0,001, BMI with C-peptide level – r = 0,450; p<0,001, BMI with HOMA index – r = 0,1504; p = 0.036. Inverse correlations of C-peptide were found with the level of P (r = -0.169; p = 0.025). This indicated the development of obese women with IR and characteristic hormonal disorders, especially hypoprogesteronemia. Clinically, this was manifested by the threat of abortion in these women, which confirmed the idea of the components of metabolic disorders in obesity. Increased glycosylated hemoglobin was associated with a burdensome family history of type 2 diabetes.

Some researchers consider leptin to be one of the means of regulating blood pressure. This confirms the presence of sOB-R in the cells of the adrenal medulla, which may indicate the regulation of leptin synthesis of vasopressors catecholamines. At the same time, high levels of catecholamines reduce leptin secretion by activating β-adrenergic receptors. Another mechanism of increasing blood pressure under the action of leptin is the activation of the SNA, which leads not only to increased heat production and energy use, but also to increased blood pressure. [10]. Thus, in women with I degree obesity, the average blood pressure was 145/80 mm Hg, while in women with III degree obesity – 163/90 mm Hg. Art. (Table III). According to the results of blood pressure monitoring in women with III degree. obesity marked an increase in SBP and DBP, which was associated with increased leptin levels. A correlation was found between the level of leptin and SBP (r = 0.50; p = 0.05) and DBP (r = 0.42; p = 0.05).

DISCUSSION

The problem of obesity is becoming increasingly important today and poses an economic, medical and social danger to human life, regardless of social or occupational distribution, place of residence, age or gender. Only 50% of the population of Ukraine has a normal weight. Instead, more than 22% are obese. According to statistics, the mortality rate of young people due to overweight is 12 times higher than for people with normal body weight.

Compared with 1960, the caloric content of food increased by 24-26%, probably due to increased consumption of refined carbohydrates, transgenic fats, sodium, potassium, fiber, non-compliance with water regime, physical activity.

Recently, the role of adipose tissue, which is considered an endocrine and paracrine organ that produces a number of hormonally active substances - adipokines (adipose derived hormones), which cause disorders of carbohydrate and lipid metabolism, regulate inflammation and immune homeostasis, has been significantly revised [1,7, 8]. The most famous of these is leptin [8].

Leptin plays an important role in the regulation of metabolism and functions of the neuroendocrine system, including the pituitary gland, hypothalamus, adrenal glands, immune system. (Fig.2).

During pregnancy, the level of leptin in the blood rises, and after childbirth and abortion – falls. During pregnancy, leptin is additionally produced in the placenta and amniotic membranes, as well as in uterine tissues, inhibiting the contraction of uterine smooth muscle [3,8].Recently, there are data on the pleiotropic effect of leptin on reproduction and pregnancy. In particular, the expression of leptin in the placenta enhances the proliferation and differentiation of trophoblast cells. With obesity and abnormal serum leptin concentrations, there are usually pathological changes in the endometrium and a low probability of implantation of a fertilized egg in the uterus, as well as the usual miscarriage [9]. There are data on the role of leptin in the development of polycystic ovaries.

Hyperleptinemia, which is associated with overweight and obesity, is considered a risk factor for chronic oligo- or anovulation, which is caused by both insulin resistance and leptin-dependent ovarian dysfunction [19] (Fig.3). Peculiarities of reproductive history and menstrual function of the main group of pregnant women were: polycystic ovary syndrome – in 40% (26 women), miscarriages – in 20% (13 women), menstrual disorders – in 20% (13 women).

There are data in the literature on the role and importance of leptin in hypertension (AH) [4, 9, 6, 10], but they are not yet final. Thus, the determination of leptin in patients with hypertension showed that with increasing its level there is not only a progressive increase in body mass index and waist circumference, but also systolic and diastolic blood pressure (BP), insulin and insulin resistance index HOMA [5]

The results of the study of the lipid profile, shown in table 1, indicate the presence of significant interdependence (from p<0.05 to p<0.01) of high levels of cholesterol, LDL, VLDL and atherogenic index from BMI of pregnant women.

The results of the study of carbohydrate metabolism, shown in table 2, indicate a significant interdependence (from p<0.05 to p<0.001) levels of blood glucose, C-peptide and HOMA-IR index from BMI of pregnant women.

Some researchers consider leptin to be one of the means of regulating blood pressure. According to the results of blood pressure monitoring in women with III degree. obesity marked an increase in SBP and DBP, which was associated with increased leptin levels.

To summarize the results of the study, a correlational relationship between the studied parameters was identified (Table I, II, III). The marked correlations are significant, at p<0,05000.

There is a correlation between:

- the level of leptin in the serum of pregnant women and the degree of obesity;
- leptin level and BMI;
- leptin level and body weight;
- leptin levels and total cholesterol;
- leptin levels and low density lipoproteins;
- leptin levels and high density lipoproteins;
- leptin levels and SBP and DBP;
- BMI and glucose level;
- BMI and C-peptide level;
- BMI with HOMA index.

Thus, adipose tissue is an endocrine and paracrine organ that produces hormonally active substances - adipokines (adipose derived hormones), which cause disorders of carbohydrate and lipid metabolism, regulate inflammation and immune homeostasis [1,7,8], the most famous of which is leptin [8].

CONCLUSIONS

1. Obese pregnant women have elevated serum leptin levels in the second trimester compared to healthy pregnant women.
2. The content of leptin in the serum of obese pregnant women correlates with the severity of the disease ($r = 0.47$; $p<0.05$).
3. Serum leptin in obese women was directly correlated with BMI ($r = 0.66$, $p<0.001$), body weight ($r = 0.29$, $p<0.05$), total cholesterol (cholesterol) ($r = 0.37$, $p<0,009$), low-density lipoproteins (LDL cholesterol) ($r = 0.33$, $p<0.05$) and vice versa with high-density particles (HDL cholesterol) ($r = - 0.37$, $p<0.02$).
4. The results of the study of carbohydrate metabolism indicate a significant interdependence: the corresponding correlation coefficients were equal to: BMI with glucose level – $r = 0.351$; $p<0,001$, BMI with C-peptide level – $r = 0,450$; $p<0,001$, BMI with HOMA index – $r = 0,1504$; $p = 0.036$. Inverse correlations of C-peptide were found with the level of P ($r = -0.169$; $p = 0.025$). This indicated the development of obese women with IR and characteristic hormonal disorders, especially hypoprogesteronemia. Clinically, this was manifested by the threat of abortion in these women, which confirmed the idea of the components of metabolic disorders in obesity.
5. Leptin is one of the means of regulating blood pressure. A correlation was found between leptin level and SBP ($r = 0.50$; $p = 0.05$) and DBP ($r = 0.42$; $p = 0.05$).

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ORIGINAL ARTICLE

EVALUATION OF SOME BIOCHEMICAL MARKERS AND THE ANTIBODY RESPONSE TO HEPATITIS B VACCINE IN HEMODIALYSIS PATIENTS

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ABSTRACT

The aim: The goal of this study was to assess the immune response to the HB vaccine (the level of anti-HBs titer), as well as the prevalence of serum creatinine, urea, CRP, and serum albumin levels, and the relationship between these and immune response to the vaccine**.**

Materials and methods: 127 patients with chronic renal disease on hemodialysis (HD) were compared to 40 healthy people in Iraqi dialysis center, Baghdad. Antibodies to the hepatitis B core antigen (anti–HBc) were detected using the ARCHITECT SYSTEM and the Anti-HBs titer, HBs Ag, Anti-HCV determined by ELISA.

Results: When compared to the poor and non-responder groups, the mean value of anti-HBs titer increased considerably in the good responder group. The good responder and control groups, on the other hand, showed no significant changes. The anti-HBs titer was found to have the strongest negative correlation with serum creatinine, blood urea, and C-reactive protein levels. There was a considerable positive connection between anti-HBs titer and albumin levels.

Conclusions: The responses of HD patients to the HB vaccine revealed the significant negative relation between serum creatinine, blood urea levels, and CRP, as well as a significant positive correlation between serum albumin.

KEY WORDS: Hepatitis B vaccination, Hemodialysis patients, Vaccination

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INTRODUCTION

Hepatitis B virus (HBV) infection and consequences are a major public health problem. Worldwide, it is estimated that over 2 billion people are infected with HBV, 350 million of whom are chronically infected; 15 percent to 25 percent of chronically infected die from chronic liver diseases [1]. Hepatitis B virus (HBV) infection is more common in hemodialysis (HD) patients. In these patients, the hepatitis B (HB) vaccine provides insufficient protection. The presence of HBV infection is relevant because infected individuals may experience severe clinical deterioration following transplantation [2]. Contact with infectious blood or body fluids transmits the hepatitis B virus [3], due to frequent, prolonged blood exposure in the extracorporeal circuit, chronic kidney disease patients who require renal replacement therapy, particularly haemodialysis (HD), have already been identified as a risk category**.** Furthermore, patients with end-stage renal disease, whether on pre-dialysis or chronic dialysis, have a weakened immune system, making them more susceptible to infection and disease [4]. Vaccination against hepatitis B (HBV) may be effective in preventing the disease. According to current recommendations, active hepatitis B virus vaccine should be given to those having advanced chronic renal disease (CKD) or individuals on dialysis [5]. A new schedule with four doses of 40 g is indicated for chronic renal disease patients who do not respond to the main vaccine. HBV infection prevention in CKD patients is a top goal, and progress has been done in recent decades [6[. In 2017 update of the United States Advisory Committee on Immunization Practices (ACIP) recommendations, the typical dose of the hepatitis B vaccine (Euvax-B) was 20 g/mL, and it should be given in four doses at 0, 1, 2, and 6 months at twice the usual dose (for a total of 40 g/mL)]7[. According to several studies conducted throughout the world, one-third of these people do not respond well to immunization. Titers of HBsAb were determined using an enzyme-linked immunosorbent assay (ELISA), antibody titers ≥10 IU/mL were considered as responders. The patients who achieved titers ≥100 IU/mL were labeled as "good responders". Patients with titers of <10 IU/mL were considered as non-responders [8].

THE AIM

The objectives of this study were to analyze immunological response to the HB vaccine (level of anti-HBs titer), as well as to determine the prevalence of serum creatinine,

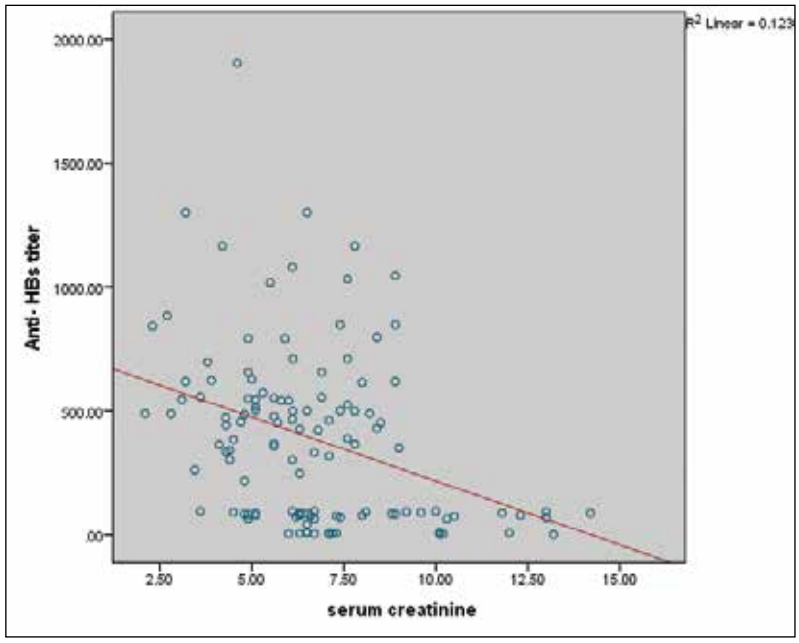

Fig 1. Anti-HBs titer and serum creatinine levels have a highly significant negative correlation. ($r = -0.351$ and $p \leq 0.01$)

urea, CRP, and serum albumin levels, and to investigate the relationship between these parameters and immune response to the vaccine.

MATERIALS AND METHODS

The study compared 127 patients with chronic kidney disease HD, who were treated in Iraqi dialysis center, Baghdad, from December 2020 to June 2021, to 40 healthy people who had been vaccinated fully. Patients on hemodialysis (HD) for chronic renal failure who participated in the study. Ethical approval for conducting this study was taken. Before beginning the study, all studied groups were negative for HCV Ab and all serologic markers of HBV. They were then given HB vaccination using a recombinant DNA HB vaccine (Euvax B) at a dose of 1.0 ml (20 g HBsAg) for the control group and 2.0 ml (40 g HBsAg) for the HD group, administered as intramuscular (deltoid) injection at 0, 1, and 6 months (according to infection control program in the Iraqi dialysis center).

The following procedures were applied to all patients and control groups:

SEROLOGICAL INVESTIGATION

- ELISA was used to determine the anti-HBs titer.
- ELISA for HBs Ag.
- Antibodies to the hepatitis B core antigen (anti – HBc) were detected by the architect system.
- ELISA detection of anti-HCV.

INVESTIGATION IN THE LABORATORY

- Renal functions, such as creatinine and urea levels in the blood.
- C-reactive protein and albumin in the blood.

Protective conversion was defined as a serum HBsAB level less than 10 mUI/m. Responders with HBsAb levels between 10 and 100mUI/ml were classified as low immune responders, while those with levels greater than 100mUI/ml were classified as high immune responders.

STATISTICAL ANALYSIS

SPSS 20 (Armonk, NY: IBM Corp. SPSS) for Windows was used to enter and analyze the data from the study. The statistical method was used to estimate the test of significance. The data was expressed using the mean and standard deviation (SD). The Chi-square test was performed to compare antibody responses between parameters. Pearson correlation analyses were used to establish the relation between parameters, all the analyses were estimated at $p<0.05$ and $p<0.01$ level of statistical significance.

RESULTS

After the full immunization regimen (0, 1, 6) was completed, there were 113 responders (88.97%) and 14 non-responders (11.03%). 33(29.21%) of the 113 respondents were poor responders, while 80 (70.79%) were good responders. Good responders showed significant increase in anti-body titer compared with non-responders and poor responders. But there were no significant differences between good responder and control group, table I. Hemodialysis patients (non-responder, poor responder and good responder for creatinine and blood urea were higher than in control group, also, non-responder and poor responder showed significant increase in serum creatinine and blood urea when compared with good responder, while there were no significant differences between non-responders and poor responders; there were significant variations between non-responders and poor responders. CRP levels in hemodialysis patients (non-responders and poor responders) were greater than those in the good responder and control groups. In addition, when compared to the control group, good responders had significantly higher CRP levels. When compared to the good responder and control groups, hemodialysis patients (non-responder and poor responder) had significantly lower albumin levels.

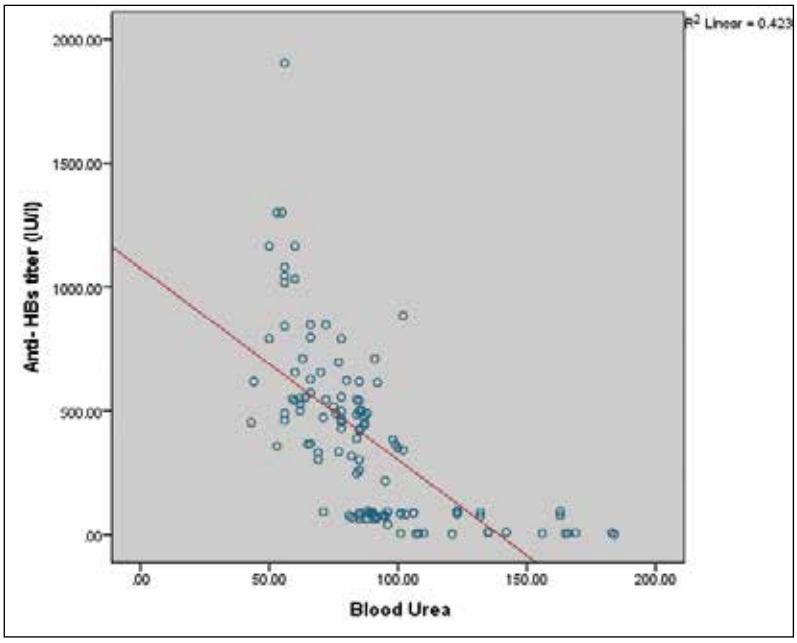

Fig 2. Anti-HBs titer and blood urea had a highly significant negative correlation (r = - 0.650 and p 0.01)

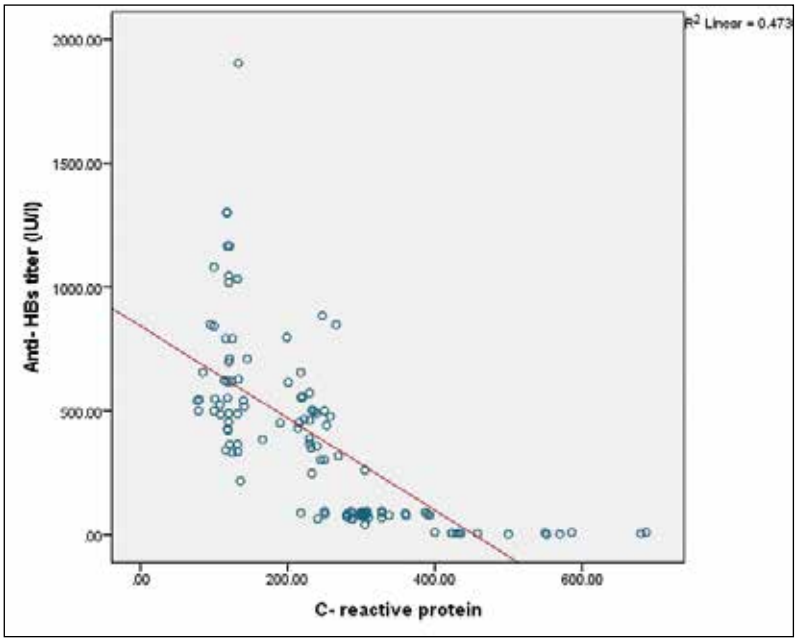

Fig 3. Anti-HBs titer and CRP had a highly significant negative correlation (r = -0.688 and p 0.01)

While there were no significant differences between the good responder and control groups, table II.

Anti-HBs titers were observed to have a highly significant negative relationship with blood urea (r = - 0.650 and p 0.01), serum creatinine (r = - 0.351 and p 0.01), and CRP (r = - 0.688 and p 0.01) in the HD subgroup. There was a significantly positive correlation between anti-HBs titer and serum albumin levels in the HD subgroup (r = 0.678 and p 0.01).

DISCUSSION

In the 1970s, HBV was very common among dialysis patients and health-care workers [10]. Universal precautions, reduced use of blood products, and erythropoietin (Epo) treatment helped to lower the prevalence of HBV among dialysis patients to less than 5% in some parts of Europe [11]. The current investigation found that three hepatitis B intramuscular (IM) doses immunization resulted in an 88.97 percent seroprotective response in both the HD and control groups. A HB vaccine seroconversion percentage of 113 (88.97%) was reached in 127 HD patients. Eighty patients (70.79 percent) have a good HB vaccination response and a high HbsAb level of >100 mIU/ml. These findings were confirmed by Al- Saran et al., who reported that 129 out of 144 (89.64 percent) patients were responders (anti-HBs 10 U/ I) and 15 out of 144 (10.4 percent) patients were non-responders (anti-HBs 10 U/ I) the researcher wants seeing how patients with HD responded to HBV immunization and what factors might influence that response [12]. Anti-HBs titer was shown to have a significant negative correlation with serum creatinine

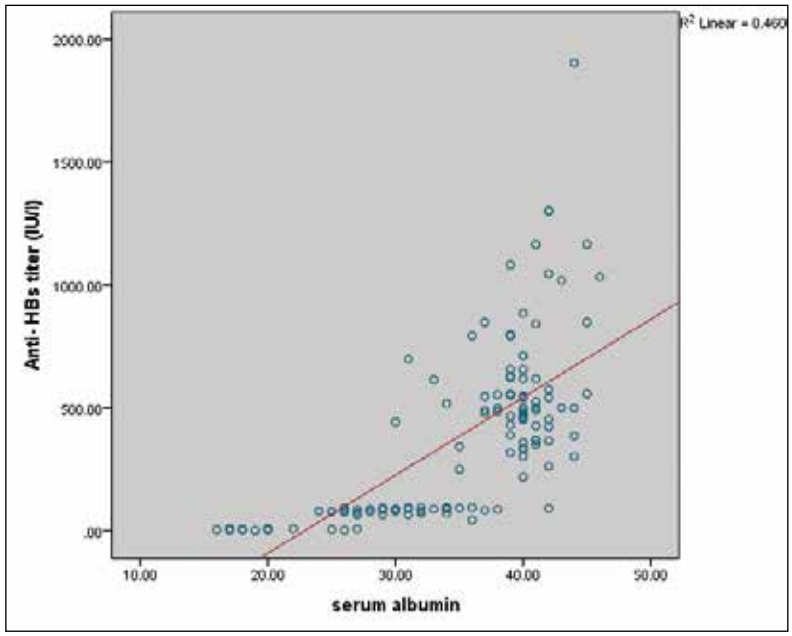

Fig 4. Anti-HBs titer and serum albumin levels had a significant positive correlation (r = 0.678 and p 0.01)

Table I. HB vaccine immune response (anti – HBs titer) among studied groups

Immune responses	Studied groups		P value
	HD group (n= 127)	Control group (n= 40)	
Non responder (titer < 10 IU/l) n = 14	5.6 ± 2.4 [a]	0	0.23 [a] 0.00 [b]
Poor responder (titer ≥ 10 IU/L) n = 33	82.03 ±11.6 [a]	0	
Good responder (titer ≥ 100 IU/L) n = 80	603 ± 291.8 [b]	594.3 ±193.9 [b]	
Total responder		113 (88.97)	
Total non responder		14(11.03)	

[a,b], Different letters refer to significant difference at P<0.05

Table II. HB vaccine immune response (anti – HBs titer) among studied groups

Parameter	Non Responder (titer < 10 IU/L) n = 14	Poor Responder (titer ≥ 10 IU/L) n = 33	Good Responder (titer ≥ 100 IU/L) n = 80	Control Group N= 40
Serum Creatinine	9.38 ± 2.192 [a]	9.17 ± 2.091 [a]	7.34 ± 1.66 [b]	0.43 ± 0.29 [c]
Blood Urea	151.97 ± 34.1 [a]	102.66 ± 24.65 [b]	84.21 ± 18.78 [c]	24.92 ± 11.63 [d]
CRP	509 ±96.52 [a]	307.27± 40.19 [b]	155.05 ± 55.98 [c]	7.78 ± 7.11[d]
Serum Albumin	21.07 ± 4.0 [a]	36 ± 4.45 [b]	39.63± 2.85 [c]	40.86 ± 3.88 [c]

The data is presented as a mean (SD).
[a,b,c,d], Different letters refer to significant difference at P<0.05

(r = -0.351 and p 0.01) and blood urea (r = - 0.650 and p 0.01). This finding corroborated the findings of Daroza *et al.*, and Ghadiani *et al.*, who found that HB immunization is less likely to produce seroconversion in the later stages of kidney illness than in the earlier stages]12-13[. Hashemi et al., on the other hand, found no link between anti-HBs titer and serum creatinine or blood urea levels [14[. In the HD subgroup, the present investigation found a strong positive correlation between anti-HBs titer and serum albumin levels (r = 0.678 and p 0.01). Brown et al., found that serum albumin level as a nutritional index was the only variable that showed importance in attaining seroconversion, and that patients with hypoalbuminemia are unable to produce sufficient anti-HBs titers.]15[In contrast, Baradana *et al.*, and Al Saran *et al.*, found no significant change in serum albumin levels between responders and non-responders to the HB vaccine (P=0.27) [16]. Anti-HBs titer and C-reactive protein were shown to have a significant negative correlation (r = -0.688 and p 0.01) in this investigation.

CRP levels in the blood are a sensitive indicator of systemic

inflammation and a risk factor for cardiovascular and atherosclerotic disease [17]. CRP levels in hemodialysis patients are eight times greater than in healthy controls, making it a strong predictor of all-cause and CVS mortality, even after a four-year follow-up period [18]. Patients with CKD have considerably lower response rates than healthy people. This observation is thought to be the result of malnutrition and inflammation, which are common in chronic kidney diseases, particularly patients on dialysis [19]. In patients with chronic inflammatory bowel illness, which is defined as a state of chronic inflammation that is frequently accompanied with malnutrition, the efficiency of the hepatitis B vaccine is lowered to a remarkably similar degree [20].

CONCLUSIONS

In chronic renal patients on HD, the immune response to the HB vaccine was adequate (anti HBs titer>100 IU/l) in 88.97% of patients versus 100% of controls, but non responder (antiHBs titer 10 IU/l) in 11.03 percent of patients. This immunological response (antiHBs) was found to be positively associated with serum albumin and negatively correlated with CRP, serum creatinine, and blood urea levels.

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Conflict of interest:

The Authors declare no conflict of interest.

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A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

FAT AND MUSCLE COMPONENTS OF BODY WEIGHT AND THEIR RELATIONSHIP WITH THE CONCENTRATION OF SERUM ADIPOKINES IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

The aim: Identify differences in indexes of body fat and muscle masses, as well as blood adipokines in patients with nonalcoholic fatty liver disease, from gender-appropriate healthy men and women.

Materials and methods: 135 patients with non-alcoholic fatty liver disease with normal, overweight and obesity and 20 almost healthy individuals for the control group were examined. Verification of the diagnosis was performed in accordance with the recommendations of the unified clinical protocol. An anthropometric examination of patients was performed according to the method, which included the determination of 48 anthropometric parameters. The formulas determined the absolute amount of adipose and muscle tissue. Levels of adipokines (leptin and adiponectin) were determined by enzyme-linked immunosorbent assay.

Results: According to Matiegka, body fat was 30.2-35.2% higher, and muscle body weight was 17.4-29.1% lower in patients with non-alcoholic fatty liver disease compared to healthy people. The concentration of leptin in the serum of patients with nonalcoholic fatty liver disease was statistically significantly higher (2.05-3.78 times) compared with almost healthy individuals. At the same time, the indicators of adiponectin concentration (1.54-1.92 times) and log A / L index (1.16-1.32 times) were lower. Correlations between changes in muscle mass and adipokines concentration have been established.

Conclusions: In addition to the known increase in body fat in non-alcoholic fatty liver disease, there has been established a significant decrease in muscle mass. A direct correlation between adiponectin concentration and an inverse correlation between leptin levels and muscle mass in patients with nonalcoholic fatty liver disease was found.

KEY WORDS: nonalcoholic fatty liver disease, adipokines, body composition, anthropometry, sarcopenia

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INTRODUCTION

Adipose and muscular tissues are the main endocrine organs that produce adipokines, myokines. These biologically active substances can be beneficial or harmful to the body by acting through the endocrine, paracrine and autocrine pathways. The pathogenesis of nonalcoholic fatty liver disease (NAFLD) is closely related to the mechanisms governing the development of obesity and metabolic syndrome. The prevalence of NAFLD increases linearly with increasing body mass index (BMI), reaching up to 14 times higher risk at a BMI of 37.5-40 kg / m2 compared to the normal weight population [1]. NAFLD is characterized by intracellular lipid deposition in hepatocytes, which is often associated with a wide range of metabolic disorders, such as dyslipidemia, hypertension, insulin resistance [2]. Lack of physical activity and sedentary lifestyle have contributed to the epidemic of obesity and NAFLD. The skeletal muscle system is the largest in the body, accounting for about 40% of human body weight [3]. Recent scientific data indicate that low muscle mass and low muscle strength and muscle muscle infiltration (myosteatosis) are associated with the severity of NAFLD. [4-7].

The content of fat in skeletal muscle highly reflects the severity of NAFLD in patients with pathological obesity. In particular, the content of muscle fat is closely related to non-alcoholic steatohepatitis (NASH) and decreases with the improvement of NASH. These data show that fatty infiltration of muscles may be a marker and a possible pathophysiological factor of NASH [8].

An important issue will be the assessment of the presence of sarcopenia in patients with NAFLD. According to the literature, people with NAFLD had a higher age-standardized prevalence of sarcopenia compared to people without NAFLD [9-11]. Among people with NAFLD, sarcopenia was more common in women than in men [12]. The European Working Group on Sarcopenia in the Elderly has identified it as "a syndrome characterized by progressive and general loss of skeletal muscle mass and strength with a risk of adverse effects such as physical disability, poor quality of life and death". All of these problems point to the potential impact of sarcopenia on mortality among NAFLD. Due to the association of NAFLD with component body composition, these patients are at risk for sarcopenic obesity. [13].

It is important to find out the factors that influence the change in body weight in NAFLD. Leptin and adiponectin are classic adipokines of adipose tissue and play an important role in the pathogenesis of obesity and metabolic complications. In skeletal muscle, leptin is involved in the absorption and oxidation of glucose and free fatty acids. In the immune system, it increases proinflammatory cytokines. The action of pro-inflammatory cytokines leads primarily to the loss of muscle mass [14]. Two transmembrane proteins, AdipoR1 and AdipoR2, have been identified as adiponectin receptors. Both receptors are mainly present in skeletal muscle and moderately in the liver [15]. Adiponectin has antilipotoxic effects that are thought to protect tissues (ie liver and muscles) from triglyceride accumulation. Decreased adiponectin levels may impair the clearance of ceramides, contributing to the accumulation of ceramides in tissues. Increased ceramide levels in the liver are associated with elevated plasma levels of ceramides in obese patients and may contribute to the development of insulin resistance at the skeletal muscle level [16]. Adiponectin is a potent cytokine that exhibits sensitizing, anti-inflammatory and antisteatotic activity to insulin in hepatocytes by increasing the oxidation of free fatty acids and reducing gluconeogenesis [17].

Leptin is another adipocytokine that is involved in the development of NAFLD. Leptin plays a dual role in NAFLD. On the one hand, it seems to play a protective role in hepatic steatosis, especially in the early stages of the disease. On the other hand, it can act as an inflammatory and fibrinogenic factor as the disease progresses. In the process of increasing visceral adipose tissue, leptin levels also increase. This should limit the increase in fat mass to avoid the formation of insulin resistance [18].

A deep understanding of the cellular mechanisms underlying the physiological processes involved in sarcopenia and NAFLD is fundamental to the development of therapeutic interventions to address the actual cause of the disease.

THE AIM

Establish differences between indexes of body fat and muscle mass, as well as blood adipokines in patients with NAFLD from gender-appropriate healthy men and women.

MATERIALS AND METHODS

The study was conducted in accordance with the requirements of the Declaration of Helsinki. The study was authorized by the Committee on Bioethics of Vinnytsya National Pirogov Memorial Medical University.

We examined 135 patients with NAFLD with normal, overweight and obesity, as well as 20 healthy individuals. The age of the subjects ranged from 36 to 60 years inclusive. Informed consent was obtained from each examined patient for the use of examination materials in scientific research.

The diagnosis of NAFLD was established according to generally accepted criteria based on the unified clinical protocol of primary and secondary (specialized) medical care "Non-alcoholic steatohepatitis" and clinical recommendations EASL-EASD-EASO for the diagnosis and treatment of NAFLD (2016), according to which the presence of fatty degeneration was determined by visualization in the absence of other causes (alcoholic, viral, autoimmune, hereditary, drug) liver damage.

To assess the presence and severity of liver fibrosis held elastometriya, which is made by the method - FibroScan (Echosens, France).

Anthropometric examination of patients with NAFLD was performed according to the method of VV Bunak, which included the determination of 48 anthropometric parameters. J. Matiegka's formulas were used to determine the absolute amount of muscle and adipose tissue, which show high reliability of the results [19].

The absolute amount of muscle tissue was determined by the formula:

$$M=L \times r^2 \times k, \quad (1)$$

where M is the absolute mass of muscle tissue (kg); L - body length (cm); r - the average value of the radii of the shoulder, forearm, thigh, shin (cm); The radius of these segments was determined by the size of the circle equal to $2\pi r$; k - a constant equal to 6.5.

The average value of the radii, except for the skin and fat layer, was calculated by the formula:

$$r = \frac{C1 + C2 + C3 + C4}{\pi \cdot 8} - \frac{(d1 + d2)/2 + d3 + d6 + d7}{80} \quad (2)$$

where C1 is the circumference of the shoulder (cm); C2 - circumference of the forearm in the upper third (cm); C3 - shin circumference in the upper third (cm); C4 - hip circumference (cm); d1 is the thickness of the skinfold on the back of the shoulder (mm); d2 is the thickness of the skinfold on the front surface of the shoulder (mm); d3 is the thickness of the skinfold on the front surface of the forearm (mm); d6 is the thickness of the skinfold on the thigh (mm); d7 is the thickness of the skinfold on the shin (mm).

The absolute amount of fat component was determined by the formula:

$$D = d \times S \times k \quad (3)$$

where: D is the total amount of fat component (kg); d is the average thickness of the subcutaneous fat layer together with the skin (mm); S - body surface (m2); k is a constant equal to 1.3.

The average thickness of the subcutaneous fat layer in women was calculated by the formula:

$$d = \frac{1}{2} \times \frac{d1 + d2 + d3 + d4 + d5 + d6 + d7}{7}, \quad (4)$$

where d1 ..... d7 - the thickness of the skin and fat folds (mm) on the shoulders, forearms, back, abdomen, thighs, legs, under the shoulder blade.

The average thickness of subcutaneous fat in men was calculated by the formula:

$$d = \frac{1}{2} \times \frac{d1 + d2 + d3 + d4 + d5 + d6 + d7 + d8}{8}, \quad (5)$$

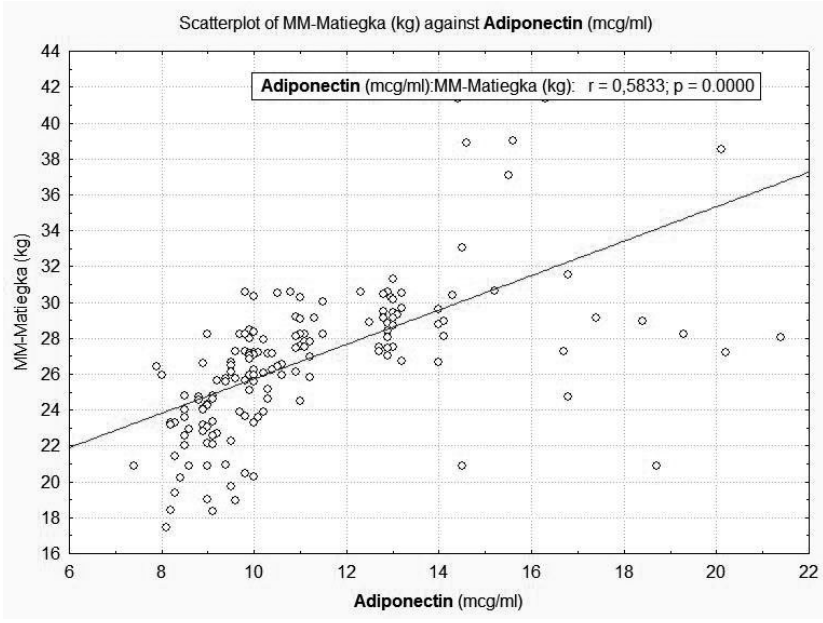

Fig. 1. The relationship between muscle mass according to the formula of Matiegka and serum adiponectin levels in patients with NAFLD.

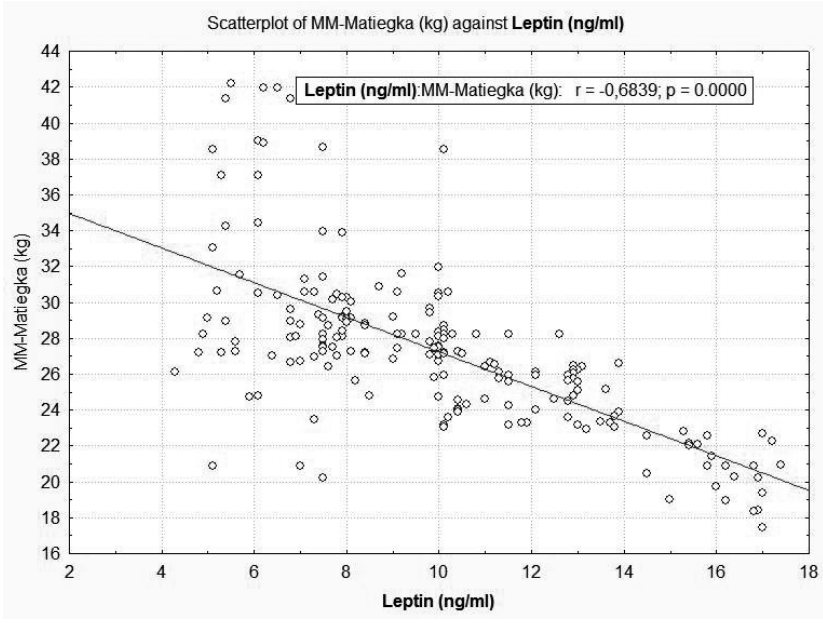

Fig. 2. The relationship between muscle mass according to Matiegka's formula and serum leptin levels in patients with NAFLD.

where d1 ..... d8 is the thickness of skin and fat folds (mm) on the shoulders, forearms, back, abdomen, thighs, lower legs, under the shoulder blade and on the chest.

The level of adipokines (leptin and adiponectin) was determined on an enzyme-linked immunosorbent assay Stat Fax 300 (USA) using the kits "Human Leptin Quantikine ELISA Kit" (R&D, USA), "Human Adiponectin ELISA" (BioVendor, Czech Republic). The ratio between the content of adiponectin and leptin was presented as a decimal logarithm (log A / L).

Statistical analysis of the obtained results was performed using the program "STATISTICA 8" from Statsoft using parametric and non-parametric methods of evaluation of the obtained results. Data are presented as median (Me) and interquartile range (Q1-Q3). The normality of the distribution was checked using the Kolmogorov – Smirnov test. Due to the asymmetric distribution, the nonparametric Mann – Whitney rank U-test was used. The statistical significance of the difference between the comparative values was considered probable at $p < 0.05$.

RESULTS

The results of the study showed that the body weight of men with NAFLD was 1.3 times higher than in the group of healthy men. The body weight of women with NAFLD was 1.5 times higher compared to the group of healthy women. There are no significant differences or trends in body length differences between healthy and NAFLD men and between women with NAFLD. The BMI of men with NAFLD was 1.4 times higher than in the group of healthy men. The BMI of women with NAFLD was also 1.4 times higher than in the group of healthy women. The body surface area in patients with NAFLD in men and women was 1.1 and 1.2 times larger than in the group of healthy men and women, respectively (Table I).

Table I. Indicators of length, weight and body surface area of healthy and patients with NAFLD, men and women (M±σ)

	Gender	Body weight (kg)	Body length (cm)	IMT	Body surface area (m2)
Healthy (n=20)	M (n=81)	74,1±9,47#	177,30±5,60#	23,53±2,40	1,91±0,12#
	W(n=144)	58,79±6,58#	165,18±4,70#	21,54±2,21	1,64±0,10#
Patients with NAFLD (n=135)	M(n=65)	96,11±8,64*	172,80±6,05	32,21±2,46*	2,13±0,09*
	W(n=47)	90,32±7,91*	171,00±6,30	30,86±2,12*	1,98±0,09*

Notes:
* – statistically significant differences ($p < 0.05$) according to the Mann-Whitney test between the respective indicators in comparison with the indicators of almost healthy individuals.
# - statistically significant differences ($p < 0.05$) according to the Mann-Whitney test between the respective indicators in comparison with the indicators of persons of the opposite sex.

Table II. Indicators of the thickness of skin and fat folds of healthy men and women with NAFLD (M±σ)

		Thickness of skin and fat folds (mm)								
	Gender	On the front surface of the shoulder	On the back of the shoulder	On the forearm	On the chest	On the lateral surface of the body	On the abdomen	On the thigh	On the shin	Under the shoulder blade
Healthy (n=20)	M (n=81)	5,21± 1,42	7,34± 1,09	4,05± 1,23	4,70± 1,08	9,81± 2,26	10,34± 2,05	12,47± 2,94	8,86± 2,12	10,79± 2,16
	W (n=144)	5,64± 1,47	6,93± 1,32	3,92± 1,38	-	11,31± 2,95	10,91± 2,04	14,18± 3,05	10,42± 2,24	10,60± 2,33
Patients with NAFLD (n=135)	M (n=65)	9,11± 1,17*	10,47± 1,43*	7,07± 0,91*	7,82± 0,94*	13,97± 1,82*	13,63± 2,06*	14,16± 1,40*	9,07± 1,29*	11,25± 2,40*
	W (n=47)	9,18± 1,21*	10,27± 1,52*	7,10± 1,02*	-	14,17± 2,17*	14,06± 2,45*	15,27± 1,58*	9,34± 1,48*	12,15± 2,68*

Note:
* - statistically significant differences ($p < 0.05$) according to the Mann-Whitney test between the respective indicators in comparison with the indicators of almost healthy individuals.

Table III. Indicators of adipocytokines in patients with NAFLD with normal, overweight and obesity, Me (Q1-Q3)

Indicator	Control	NAFLD			
	Normal body weight (n=20)	Normal body weight (n=35)	Excess body weight (n=35)	Obesity 1 degree (n=47)	Obesity 2 degree (n=18)
Adiponectin (μg / ml)	17,23 (13,12-18,85)	16,50 (14,53-18,15)	11,20* (10,18-12,96)	9,45* (8,90-10,00)	9,00* (8,48-9,25)
Leptin (ng / ml)	4,26 (3,83-5,25)	5,76* (5,13-6,20)	8,55* (7,50-10,00)	12,50* (11,58-13,00)	16,10* (15,40-16,90)
Index log A/L	3,62 (3,40-3,74)	3,47* (3,38-3,52)	3,13* (3,01-3,23)	2,87* (2,85-2,91)	2,74* (2,72-2,77)

Note. * - statistically significant differences ($p < 0.05$) according to the Mann-Whitney test between the respective indicators in comparison with the indicators of almost healthy individuals.

The thickness of skin and fat folds on the front and back of the shoulder, forearm, back, abdomen, thighs, lower legs, under the shoulder blade in men and women with NAFLD, statistically significantly greater than in healthy men and healthy women, respectively ($p < 0,001$). The thickness of the skin and fat folds on the chest in healthy men was statistically significantly lower ($p < 0.001$) than in patients with NAFLD (Table II).

We determined the average value of the radii of the shoulder, forearm, thigh, lower leg in the areas of greatest muscle development (except for the skin-fat layer) was found in men and women with NAFLD, statistically significantly smaller ($p < 0.05$) than in the corresponding gender of healthy men and women.

Thus, the average value of the radii of the shoulder, forearm, thigh, lower leg in the areas of greatest muscle

development (except for the skin and fat layer) in men with NAFLD was 4.66 ± 0.16 cm, and in women with NAFLD, was 4.62 ± 0.13 cm. In the group of healthy men, the average radius of the shoulder, forearm, thigh, shin was 5.51 ± 0.37 cm, and in women - 5.09 ± 0.32 cm.

Based on the above indicators, we determined the component composition of body weight in patients with NAFLD. It was found that the body weight according to Matiegka in men and women with NAFLD was statistically significantly higher ($p < 0.001$) than in the corresponding healthy men and women. Thus, in men with NAFLD, it was 16.23 ± 2.15 (kg), while in healthy ones - 10.52 ± 2.44 (kg). In women with NAFLD, it was 14.80 ± 2.47 (kg), while in healthy - 10.33 ± 1.98 (kg). There was no statistically significant difference in body fat mass between men and women.

According to Matiegka's formula, muscle mass in men and women with NAFLD was statistically significantly lower ($p < 0.05$) than in the corresponding healthy men and women. Thus, in men with NAFLD, it was 25.01 ± 1.95 (kg), while in healthy - 35.28 ± 2.26 (kg). In women with NAFLD, it was 23.15 ± 1.32 (kg), while in healthy - 10.33 ± 1.98 (kg). Moreover, in healthy men compared to healthy women, muscle mass was greater by 20.5% ($p < 0.05$).

According to the results of the study, the component composition of body weight in NAFLD changes. Compared to healthy men with NAFLD, the body fat mass according to Matiegka's formula was 35.2% higher, and the muscle mass in men, on the other hand, was 29.1% lower. Compared to healthy women with NAFLD, Matiegka's body fat mass was 30.2% higher, while women's muscle mass was 17.4% lower.

We observed that the concentration of leptin in the serum of patients with NAFLD was statistically significantly higher (2.05-3.78 times) compared with almost healthy individuals. At the same time, the indicators of adiponectin concentration (1.54-1.92 times) and log A / L index (1.16-1.32 times) were lower, respectively (Table III).

Inversely strong correlations ($r = 0.71$, $p < 0.001$) were found between muscle mass according to the Matiegka formula and body mass index in men and women with NAFLD.

We found a direct medium-strength relationship ($r = 0,58$; $p = 0,0000$) between serum adiponectin concentration and muscle mass index of patients with NAFLD (Fig. 1).

Correlation analysis revealed the presence of the inverse mean strength of leptin dependence ($r = 0.68$; $p = 0.0000$) between Matiegka's muscle mass and leptin concentration (Fig. 2).

DISCUSSION

Excessive accumulation of fat, especially in the abdominal area, is the cause of hormonal and metabolic disorders that lead to the development of obesity-related diseases. Recent studies have shown that sarcopenia and NAFLD have similar pathophysiological characteristics [20]. The pathogenesis of sarcopenia and NAFLD has common mechanisms: insulin resistance, increased inflammation, skeletal muscle secretion of myokines, myostatin, decreased adiponectin, vitamin D deficiency and lack of physical activity [21].

Adipocytokines modulate various aspects of skeletal muscle metabolism, so any change in cytokine secretion during obesity can negatively modulate insulin sensitivity [22].

Serum leptin had a positive correlation with anthropometric parameters and HOMA-IR, while serum adiponectin and adiponectin-leptin ratio were inversely correlated with these variables. It is also suggested that increased oxidative stress of skeletal muscle may link obesity and chronic inflammation. The mechanisms underlying skeletal muscle metabolism in obesity remain unclear. Regulation of skeletal muscle mass is a complex process that involves the precise coordination of several metabolic and intracellular signalling pathways, which ultimately affects the dynamic balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB).

Skeletal muscle mass maintains a balance between protein synthesis, regenerative capacity, and regulation of skeletal muscle function. Several researchers [23] have described the negative effects of sarcopenia in liver cirrhosis. Skeletal muscle contains the main supply of protein in the human body. Scientists have linked the development of sarcopenia in liver cirrhosis to impaired urea cycle activity, which begins in the liver mitochondrial matrix, leading to hyperammonemia [24].

Sarcopenic obesity in combination with progressive loss of skeletal muscle mass adversely affects a person's metabolic status, leading to a decrease in quality of life, the development of cardiovascular disease [25].

The causes of sarcopenia have been studied for many years, several hypotheses have been put forward; however, the mechanisms that cause sarcopenia are still debated. Although physical changes begin to manifest in old age, recent studies have shown that changes at the cellular and molecular levels precede the symptoms of sarcopenia. The search for effective methods of treatment of NAFLD on the background of metabolic syndrome is a priority of modern medicine.

CONCLUSIONS

Along with the known increase in body fat in non-alcoholic fatty liver disease, a significant decrease in muscle mass was found. A direct correlation between adiponectin concentration and an inverse correlation between leptin levels and muscle mass in patients with nonalcoholic fatty liver disease was established.

Our results allow to identify new potential therapeutic targets.

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ORIGINAL ARTICLE

MOLECULAR DETECTION OF THE TWO VIRULENCE GENES *HWP1* AND *ALS1* IN *CANDIDA* SPECIES ISOLATED FROM ONYCHOMYCOSIS

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ABSTRACT

The aim: This article was aimed to assess the prevalence of *Candida* species causing nail infections and to investigate the most important virulence genes that cause adhesion.
Materials and methods: Samples were collected from General Hospital and the outpatient's clinics in Thi-Qar Province, southern Iraq, during the period from January 2021 to May 2021. Samples were inoculated on Sabouraud Dextrose Agar plates containing 0.5 mg chloramphenicol, then Petri dishes were incubated at 30°C under aerobic conditions and observed for *Candida* species growth for 24-72 hrs. All isolates were identified by morphologic and chromatic properties on CHROMagar and by Api *Candida*.
Results: Twenty five isolates were obtained from hundred samples, which belong to five species of the genus *Candida*, distributed in percentage 9(36%), 4(16%), 4(16%), 4(16%) and 3(12%) of *C. glabrata, C. krusei, C. albicans, C. famata* and *C. tropicalis* consecutively and one isolate was not identified. Monoplex PCR technique was used to detect virulence genes (hwp1 and *als1*) of *Candida* species. Four *Candida albicans* isolates (16%) were carried of both hwp1 and *als1* genes.
Conclusions: The virulence gene *(hwp1)* can be relied upon in molecular detection as a special diagnostic tool of *Candida albicans* species except other types causing Candidal onychomycosis.

KEY WORDS: Onychomycosis, CHROMagar, *Candida tropicalis*

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INTRODUCTION

The fungal infections of the nails (onychomycosis) were caused by dermatophyte, *Candida* species and non-dermatophyte moulds [1]. Onychomycosis causes coloring, onycholysis, and thickening of the nail plate, usually manifests as a white or yellow-brown staining of the nail or green, black, and violaceous [2-3]. Onychomycosis is more common in older, in those having abnormal nail morphology, immunodeficiency, and genetic factors, nail trauma or psoriasis, wearing occlusive footwear, participation in sports like running or swimming, and a history of fungal infection elsewhere on the body [3-4]. *Candida* species are the source of fungal infections as a superficial mycosis like onychomycosis [5]. *Candida* species pathogenicity is attributed to a number of virulence factors, including the ability to adhere to human buccal epithelial cells (HBECs) and endothelial cells, the ability to transit from bud to hyphae (morphogenesis), a *C. albicans*-specific phenomenon, the production of lytic enzymes (proteinases, phospholipases, and hemolysis), phenotypic switching and biofilm formation [6]. The hyphal wall protein (hwp1) is a main adhesion protein, (transglutaminase substrate) which functions as an adhesion, are important for the pathogenicity, commonly expressed on the germ tube and hyphal surface of Candida species [7-8]. Also, the eight *ALS* genes (*ALS1* through *ALS7* and *ALS9*) are code for the large cell surface glycoproteins, some of which aid in host surface attachment [9-11] *Candida albicans* proteins in the agglutinin like Sequence (Als1) family are among the best-studied fungal adhesions [12]. The most important adhesions found on the fungal cell wall in *Candida* species are als1 and hwp1 [13-14]. Adhesion proteins such as als1-7, als9, hwp1, eap1, and pga1 assist yeast cells to adhere to the host surface [13].

THE AIM

This article was achieved to assess the prevalence of *Candida* species responsible for pathogenicity and to investigate the most important virulence genes that cause adhesion.

MATERIALS AND METHODS

Samples were collected from individuals with nail infections and healthy individuals (control), of both sexes within different age groups, ranging between (16–63 years). These samples were collected from Al-Rifai General Hospital and the outpatients' clinics in Al-Shatra district, Thi-Qar Province, during January 2021 - May 2021. Samples were cultured on Sabouraud Dextrose Agar plates, containing 50 mg chloramphenicol, then all *Candida* species colonies were purified by subculture on Sabouraud Dextrose Agar and incubated at 30°C for 24-48 hrs. The rapid isolation and identification of *Candida* species directly from mixed cultures was achieved by using CHROMagar *Candida* and API *Candida*. DNA of *Candida* species was

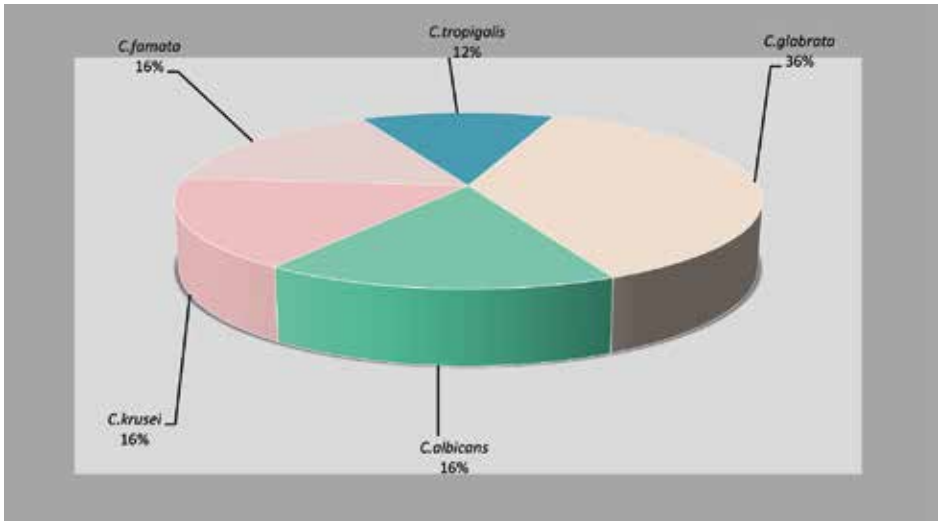

Fig. 1. The percentage of each Candida spp from the total Candida isolates

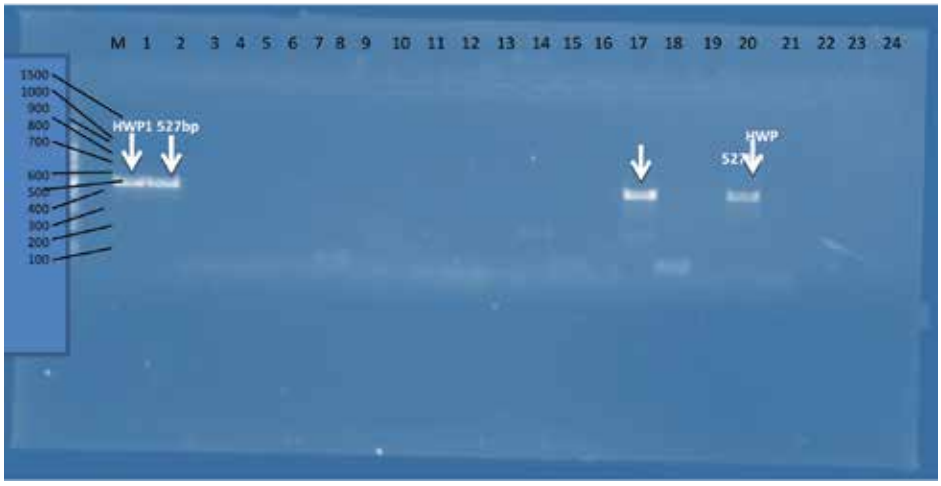

Fig. 2. Gel electrophoresis of amplified PCR products ofHWP1gene (527bp) of Candida species isolates on (1.5%) agarose gel for 1.5 hours and 60 V

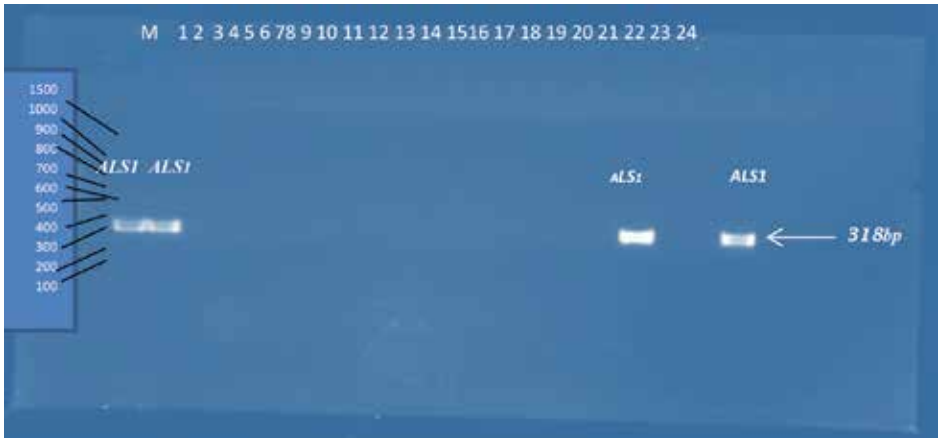

Fig. 3. Gel electrophoresis of amplified PCR products ofALS1gene (318bp) of Candida species isolates on (1.5%) agarose gel for 1.5 hours and 60 V

extracted of all isolates by a modified version of the method by [15]. For the multiplication of genes, the components required were mixed together to get on final volume (25 µl). *Hwp1* and *als1* genes were amplified in thermocycler Analytic Jena, Germany. The first cycle of Initial denaturation was for 4 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 sec and annealing at 52 °C for 1min. Extension step was 72 °C for 2 min and final extension was at 72 °C for 5 min. Agarose gel was prepared by adding 1.5 gm agarose powder to 100 ml of TBE buffer prepared. The amplified products of PCR were observed when there was DNA band equal to the target product size and visualized by ethidium bromide stain.

RESULTS

ISOLATION AND IDENTIFICATION OF CANDIDA SPP

All samples were cultured on a Sabouraud Dextrose agar medium with 0.5mg chloramphenicol, and again growing colonies was streaking on Sabouraud Dextrose agar to

Table I. The HWP1and ALS1 genes amplification results by Monoplex PCR

N. isolates	Candida spp	HWP1genes amplification	ALS1genes amplification
4	*C. albicans*	4(100%)	4(100%)
3	*C. tropicals*	No. identification	No. identification
4	*C. krusei*	No. identification	No. identification
9	*C. glabrata*	No. identification	No. identification

get pure isolates of *Candida*. All isolates were identified by morphologic and chromatic properties of colonies on the cultural media Chromogenic agar. Diagnostic results for *Candida* species` isolates by CHROMagar were less accurate than the diagnosis by Api *Candida* system, which gave a high accuracy up to 100%. The number of isolates obtained from the total samples number was 25 isolates, belonging to 5 species of the genus *Candida*, distributed in percentage 9(36%), 4(16%), 4(16%), 4(16%) and 3(12%) of *C. glabrata, C. krusei, C. albicans, C. famata* and *C. tropicalis* respectively as shown in figure (1) and one isolate was not identified.

HWP1 GENE AMPLIFICATION

Scanning was performed in all 25 *Candida* species isolates to detect *hwp1* gene presence. In a Monoplex PCR assay for *hwp1* gene, the present study results revealed that *Candida* species isolates carried hwp1 gene in 16% that equal to target 572 bp product sizes, (figure 2), while the *hwp1* gene was carried in a percentage 100% of *C. albicans* isolates.

ALS1 GENE AMPLIFICATION

Candida DNA molecules extracted from yeast isolates, were subjected to PCR technique in a Monoplex pattern to detect ALS1 gene. The results of the present study showed that *ALS1* gene band detected at 318 bp region, figure (3). The results detected that out of 25 isolates of all *Candida* species, the gene *ALS1* was carried in a percentage (16%), while the gene *ALS1* was carried in a percentage 100% of *C. albicans* isolates.

Four *Candida albicans* isolates (16%) were carried of both hwp1 and als1 genes and all the other isolates of *Candida* species were not carried for *hwp1* and *als1* genes as in table I.

DISCUSSION

Onychomycosis is the most common infectious disorder of nails and accounts for approximately 50% of all nail disorders [16]. Onychomycosis considers an important public health problem because of the high prevalence and pathogenicity associated with the following conditions: psychosocial effects, occupational discomfort, and spreading of infection to other individuals, permanent damage to nails and high cost of treatment [17]. The results indicated that the *Candida* species isolates were represented in 32% of infected individuals and in 18% of healthy individuals, quite high percentage compared to infected individuals. This study suggests that the high infections of healthy individuals are likely due to the fact that the fungal factor *Candida* was carried in their nails just before infection occurred. Also, healthy people in fact have Candidal onychomycosis infections, but it has not been diagnosed by a dermatologist due to lack of the person's education and awareness for nail health. In the present study the infected individuals with Candida onychomycosis showed isolation rates 32% (16 isolates from fifty infected individuals). This result agrees with the results obtained by Ding *et al*, 2017, that showed the isolation rate of *Candida* spp was 31.5% from infected individuals [18].

In India, other study conducted by Rather *et al*, 2021, had 384 samples which gave positive results of *Candida* spp isolation in percentage 26.3% [19]; on the other hand, Araiza-Santibánez *et al*., 2016 found that the isolation rate of *Candida* species was low 17.4% (24 isolates obtained from 138 patients) [20]. In contrast, Meireles *et al*., 2008, Brazil, conducted the prospective study and other retrospective study which showed that *Candida* species were isolated in high rates 64.4%, 84.3% (59 and 127 cases of onychomycosis respectively) [21]. In the present study, individuals infected with *C. albicans*, were having high pathogenicity during the clinical examination where that all *C. albicans* isolates were carrying the *hwp1* gene. These findings correspond to results of Fan's *et al*, 2013 that showed *C. albicans* pathogenesis is closely related to *hwp1* gene [22]. The results of Tsuchimori *et al*., 2000, indicated that the *hwp1* gene product plays great role for the *C. albicans* virulence [23]. As well, the critical role in biofilm formation of hwp1 was mentioned [24]. In our study, *hwp1* gene amplified for non-*C. albicans* isolates represented by *C. tropicalis, C. glabrata, C. krusei* and *C. famata*; any efficacy of *hwp1* gene between non-*C. albicans* isolates was not revealed. All four isolates of *Candida albicans* that were carriers of the *hwp1* gene, were also carriers of *als1* gene, and this indicates the existence of a relationship between both *hwp1* and *Als1* genes and virulence from another perspective. Molecular detection in the present study did not reveal any nucleotide sequences of *ALS1* gene amplified for non-*C. albicans* isolates represented by *C. tropicalis, C. glabrata, C. krusei* and *C. famata* and this could be due to the need of more samples required for analysis.

CONCLUSIONS

Among the *Candida* species that cause Candidal onychomycosis, *Candida glabrata* isolates obtained from nail infections

were the most common. Molecular detection revealed that only all *Candida albicans* isolates carry the *hwp1* gene as well as the *als1* gene at the same time, while non-*C. albicans* isolates did not reveal any amplified genes *hwp1* and *als1*.

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ORIGINAL ARTICLE

BEREAVEMENT AND COVID-19: PREVALENCE, COMORBIDITY, AND ASSOCIATED FEATURES AMONG UKRAINIAN SAMPLE

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ABSTRACT

The aim: To examine the features of experiences of bereavement reactions and the severity of comorbid mental health problems in persons who lost loved ones during the pandemic.

Materials and methods: 191 volunteers aged 18-60 years participated in this study. All participants were tested during 2018-2020. To assess the mental health problems was used International Neuropsychiatric Interview (MINI) and a set of IAPT scales; to the psycho-emotional distress severity associated with loss of loved ones – Inventory of Complicated Grief, with verification of symptoms of complicated grief based on an independent assessment of two experts; and the quality of life satisfaction was assessed by the Quality of Life Enjoyment and Satisfaction Questionnaire, Short Form.

Results: The results show an association between the severity of symptoms of depression, general anxiety, social avoidance, and loss during the pandemic. The level of psycho-emotional distress was associated with the experience of bereavement as a traumatic event for a group of participants who experienced loss during the pandemic and, at the same time, before the pandemic was associated with the severity of depression. Regardless of the time of loss, people who demonstrated signs of complicated grief have a more pronounced comorbid psychopathology, a higher level of psycho-emotional distress, and a lower level of satisfaction with life quality.

Conclusions: It has been established that a person's ability to experience loss as a traumatic experience and grieve during the pandemic is modified with the context of the life situation and reflects those challenges that impose quarantine restrictions

KEY WORDS: bereavement; complicated grief; COVID-19 pandemic; depression; anxiety disorders

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INTRODUCTION

The grieving process, despite specific patterns, can vary significantly from person to person with a diverse range of feelings that cannot be classified as "right" or "wrong," and often exacerbates feelings of emotional and social distress during the recovery period from a bereavement. Peculiarities of the relationship with the person lost, life-long coping strategies, and available support resources from the environment play an essential role in the grieving process [1-3]. At the same time, the circumstances in which the world has found itself since 2020 force us to reconsider aspects of grief and its comorbidities, taking into account the restrictions imposed by the COVID-19 pandemic.

Common psycho-emotional distresses associated with grief may include feelings of control loss, insecurity, excessive guilt, excessive anxiety and fear of death, or inability to "distance" oneself from thoughts of a loved one and causes of their death.

A systematic review by M. Stroebe and H. Schut [4] explains that forced socioeconomic changes and restrictions in response to the prevalence and mortality of the pandemic are themselves modifiers that increase people's vulnerability to distress and propensity to social isolation, that inhibits mourning by the traditional way.

In the same way, limits of communication and opportunity to care at a distance, including by staying loved ones in inpatient units and restricting to funeral services in fatal cases, can increase social discontent, anger or exacerbate social injustice [2; 4-6]. Considering the analysis of the concepts of grief in the psychological theories can be pointed out that the grieving process will reflect the context of social and cultural aspects of the individual's environment at the time of bereavement; the time elapsed since the bereavement and duration of pathopsychological symptoms accompanying this journey [7-10]. Among the risk factors of complicated grief in the context of the COVID-19 pandemic should be noted a medical history of mental disorders (including drug or alcohol use), socioeconomic factors (social isolation by pandemic restriction, living alone, loss of a guardian, or financial security), features of available support from relatives (including the participation of relatives in decision-making on treatment) and a specific family tradition of perception of the death [6; 11-14].

Studies of factors associated with an increased risk of complicated grief published before the pandemic have shown an association with anxiety disorders, including PTSD, panic disorder, and generalized anxiety disorder,

and a lower quality of life compared to people without anxiety disorders in their medical history [15-18]. At the same time, one of the vulnerabilities, in our opinion, may be a tendency to high levels of social anxiety and the presence of social anxiety disorder in particular. Thus, social isolation, restrictions on direct support from the closest environment, and access to spiritual, medical, or psychological professional help are expected to increase the risk of complicated grief as a way of processing traumatic experiences.

The current study will provide an initial assessment of the prevalence and severity of grief-related mental health disorders among people who have suffered during the pandemic. We expect that will increase knowledge about the need for psychological support and treatment and may form the basis for developing preventive measures appropriate to the current situation.

THE AIM

The study aims to analyze comorbid pathopsychological changes in persons who lost loved ones during the COVID pandemic.

MATERIALS AND METHODS

The sample included people who sought psychotherapeutic help by psycho-emotional discomfort of bereavement from 2018 to 2020. Data obtained during diagnostic interviews (with the informed consent of the participants) were used for this study. Diagnostic interviews in the period between May and December 2020 were conducted online.

The criteria for inclusion: men and women aged 18 to 60 years who had experienced a bereavement and didn't receive medical treatment at the time of consulting. Exclusion criteria included a medical history of disorders such as schizophrenia, bipolar disorder, psycho-organic disorders, and intellectual disabilities, as well as the presence of persistent mental illness associated with substance use in the previous 12 months. All participants confirmed the absence of active COVID disease symptoms.

The interviews collected socio-demographic data, information on the history of previous losses and grief, and actual bereavement experience. A diagnostic interview, The MINI: International Neuropsychiatric Interview by Sheehan D.V. and Lecrubier Y. (adapted by I.Ushtan, 2011), was used to screen for mental status, and questions from Inventory of Complicated Grief: measures maladaptive responses to loss (1995) were used to assess the severity of psycho-emotional distress associated with loss as screening of complicated grief. This questionnaire has not been adapted and validated in Ukraine, imposing certain restrictions on the evaluation criteria. The questionnaire was tested in the English sample ($\alpha = 0.94$; test-retest reliability = 0.80) and demonstrated high reliability and validity in diagnosing signs of complicated grief [19; 20]. We translated the English version of the questionnaire and had done a reverse translation to verify the adequacy of the content of the proposed statements. After that, we used the diagnostic criteria proposed in DSM-5 and ICD-11 based on an independent diagnostic assessment by two experts to verify the signs of complicated grief.

Assessment of the manifestations and severity of comorbid conditions was performed following the NICE recommendations based on a set of IAPT scales [21]. Q-LES-Q-SF: Quality of Life Enjoyment and Satisfaction Questionnaire, Short Form [22] were used to assess life satisfaction.

Differences between groups on continuous variables used two-way t-tests for categorical variables - Chi-square ($\chi 2$) test. The mean values of scales cores were compared between groups using ANOVA with post hoc tests (Tukey'smethod). Multiple linear regression analyses were conducted for the level of psycho-emotional distress, quality of life enjoyment and satisfaction, and the severity of social phobia symptoms. Statistical analyses were conducted using SPSS Version 23.0 (SPSS Inc., 2019).

RESULTS

The study sample was 191 people, 152 (79,84%) of middle-aged women 31,2 years. 92 participants (48,16%) experienced bereavement during the COVID-19 pandemic; among them, 53 (30,18%) reported a loss of loved ones due to complications of COVID-19. The period from the moment of loss averaged eight months. Given that at least six months after the bereavement, signs of grief were the leading cause of psycho-emotional discomfort, 57 people (29,84%) showed symptoms that met the criteria of complicated grief. At the time of the interviewing, 60 people (31,41%) lived alone, including mourners who found themselves in the quarantine zone imposed in 2020 and could not live with relatives. At the same time, a comparative analysis of socio-demographic indicators of age, gender, education, availability of social support (as living nearby), and duration after bereavement did not reveal significant differences ($p<0,05$) between the group of people who loss of loved ones before pandemics and those who lost during this period.

Table I presents the prevalence of mood and anxiety disorders, current symptom severity, and social impairment in subjects who lost loved ones before and during the pandemic.

The results did not demonstrate a significant difference in the prevalence of signs of complicated grief by groups. The results showed that the prevalence of mental health problems in the last five years also had no statically significant distribution.

The comparative analysis revealed statistically higher levels of severity depressive and anxiety symptoms ($p<0,01$), a stronger tendency to avoid social interaction ($p<0,05$), and a lower level of life satisfaction ($p<0,05$) in the group of the bereaved during the pandemic.

In addition, analysis of the linear regression results revealed the impact of the grieving level as a traumatic event on the higher level of psycho-emotional distress for the

Table I. Compared characteristics of the bereaved during and before the pandemic.

	Lost during pandemic (n=92)	Lost before pandemic (n=99)		df	Analyses (*p<0,05)
Mental health disorder (with in the past five years), % (n)					
MDD	68,5 (63)	60,6 (60)	χ2 = 1,14	1	0,285
Generalized anxiety disorder	18,5 (17)	11,1 (11)	χ2 = 1,46	1	0,227
Social anxiety disorder	40,2 (37)	46,4 (46)	χ2 = 1,54	1	0,283
Panic disorder	13, 04 (12)	7,07 (7)	χ2 = 2,33	1	0,127
Specific phobia	7,61 (7)	-	-	-	-
Posttraumatic stress disorder	22,8 (21)	20,2 (20)	χ2 = 0,005	1	0,946
Alcohol abuse	2,17 (2)	6,06 (6)	χ2 = 0,143	1	0,706
Alcohol dependence	-	-	-	-	-
Current comorbid complicated grief					
CG	33,69 (31)	26,26 (26)	χ2=0,368	1	0,544
Current symptom severity and social impairment, mean (SD)					
PHQ-9	16,32 (3,04)	15,16 (2,38)	t = 2,931	172,24	0,004*
GAD-7	11,22 (2,88)	9,92 (3,49)	t = 2,814	186,53	0,005*
SPIN	26,65 (10,05)	23,26 (12,01)	t = 2,120	187,02	0,035*
IES-R	24,58 (3,28)	24,36(3,61)	t = 0,147	188,93	0,649
W&SAS	34,29 (3,16)	33,85 (4,09)	t = 0,825	182,83	0,411
Q-LES-Q-SF	28,56 (4,56)	30,13 (5,77)	t = -2,087	184,28	0,038*

Table II. Compared characteristics of the bereaved participants due to COVID-19 and other reasons during the pandemic

	Bereaved -COVID (n=39)	Bereaved +COVID (n=53)		df	Analyses (*p<0,05)
Current comorbid complicated grief					
CG	38,46 (15)	30,18 (16)	χ2 = 0,574	1	0,718
Current symptom severity and social impairment, mean (SD)					
PHQ-9	16,89 (2,87)	15,09 (3,12)	t = 1,58	85,53	0,118
GAD-7	11,10 (3,16)	11,32 (2,69)	t = -0,35	73,90	0,729
SPIN	27,76 (10,52)	25,83 (9,72)	t = 0,90	78,15	0,369
IES-R	24,77 (3,29)	24,45 (3,41)	t = 0,90	85,61	0,646
W&SAS	34,49 (3,16)	34,15 (3,07)	t = 0,49	78,58	0,620
Q-LES-Q-SF	28,33 (5,48)	28,73 (3,79)	t = -0,39	63,64	0,695

-COVID – bereaved participants who lost loved ones for other reasons during the pandemic; +COVID – bereaved participants who lost loved ones due to COVID-19 during the pandemic

group of participants who suffered a bereavement during the pandemic (B = 0,595, t = 3,261, p<0,01). In contrast, this indicator in the group who experienced bereavement before the pandemic was associated with the severity of depressive symptoms (B = 0,524, t = 1,754, p<0,05).

The lower level of satisfaction with the quality of life in the group of people who suffered losses during the pandemic showed an association with the severity of generalized anxiety disorder symptoms (B = -0,191, t = -2,475, p<0,05) and the level of traumatic events impact (B = -0,502, t = -3,283, p<0,01). In the group that had suffered a loss before the pandemic, there was an association with the levels of depressive symptoms (B = -0,134, t = -2,284, p<0,01) and the level of grief as a traumatic event (B = -0,213, t = -2,026, p<0,05).

In both groups, the level of social avoidance and distress in social communications was associated with the severity of the generalized anxiety disorder symptoms (during - B = 1,875, t = 7,401, p<0,001; before - B = 1,963, t = 8,031, p<0,001), depressive symptoms (during - B = 1,314, t = 5,644, p <0,001; before - B = 0,954, t = 2,307, p<0,05), and the level of traumatic events impact (during - B = 0,467, t = 2,229, p<0,05; before - B = 0,474, t = 2,023, p<0,05).

Another factor we considered in the study was the death of a loved one from the coronavirus infection. When

Table III. Clinical correlates current symptom severity and social impairment of the bereaved with complicated grief and without.

	During pandemic		Before pandemic		ANOVA F value	p	Tukey's post-hoc tests*
	CG n=31	NoCG n=61	CG n=26	NoCG n=73			
	1	2	3	4			
PHQ-9	19,72 (2,07)	14,76 (1,89)	17,38 (2,80)	14,36 (1,60)	39,18	,000 ,003 ,002 ,000	1 vs 2, 4: p<0,001 1 vs 3: p<0,01 2 vs 4: p<0,01 3 vs 4: p<0,001
GAD-7	13,96 (2,56)	9,97 (2,08)	14,27 (3,17)	8,38 (1,96)	40,98	,000 ,000	1 vs 2, 4: p<0,001 3 vs 2, 4: p<0,001
SPIN	37,38 (9,32)	21,71 (5,75)	39,81 (10,74)	19,97 (4,78)	52,83	,000 ,000	1 vs 2,4: p<0,001 3 vs 2,4: p<0,001
IES-R	25,38 (3,08)	24,71 (3,24)	26,73 (3,26)	23,73 (3,20)	5,98	0,001	3 vs 2, 4: p<0,001
W&SAS	34,93 (3,52)	34,00 (2,96)	37,00 (2,15)	32,73 (4,05)	6,81	,002 ,000	3 vs 2: p<0,01 3 vs 4: p<0,001
Q-LES-Q-SF	27,18 (4,07)	28,46 (5,69)	24,88 (4,10)	32,00 (5,11)	10,27	,004 ,016 ,003 ,000	1 vs 4: p<0,01 2 vs 4: p<0,05 3 vs 2: p<0,01 3 vs 4: p<0,001

CG – with complicated grief; NoCG – without complicated grief
* - only post-hoc pairs who showed significant difference included

comparing casualties during the pandemic, found no statistically significant differences in the prevalence of current comorbid disorders and concomitant psycho-emotional distress due to a bereavement caused by complications of COVID-19 (Table II).

Further analysis of the severity of current comorbid disorders and concomitant psycho-emotional distress was focused on subgroups of participants with distribution according to signs that meet the criteria of complicated grief (Table III).

The results of post-hoc tests indicate that people who show signs of complicated grief, regardless of the period when they lost loved ones, have a more pronounced comorbid pathology, higher levels of psycho-emotional distress, and lower levels of satisfaction with the quality of their lives.

DISCUSSION

Although grief following the death of a significant person is a normal human response, it should be considered by the numerous psychological, social, economic, and medical impacts of the COVID-19 pandemic. The studies of the prevalence of this phenomenon indicate from 10% among adults after non-traumatic loss to 49% after traumatic loss [23]. According to the theoretical model of the study, the presence of signs and symptoms that indicate prolonged grief reactions were considered in the context of the pathogenesis of complicated grief. We expected that the prevalence of complicated grief signs in the pandemic would be statistically higher but didn't confirm our hypothesis within the study groups.

We assume that one of the possible reasons for absent of the statically significant difference is diagnostic errors at previous visits to various specialists for clinically significant signs of major depressive disorder, PTSD, and other anxiety reactions to bereavement, manifested during the last six months. Another possible reason is to diagnose the major depressive disorder and/or PTSD, which could justify hospitalization when seeking medical care from public mental health services.

Statistically higher levels of severity of comorbid symptoms in the group who experienced bereavement during the pandemic may be due to the peculiarities of quarantine restrictions. The challenges posed by the COVID context (limited psychological and social resources, family problems, relationship and communication issues, physical and mental health problems, etc.) reduce a person's resilience and exacerbate depressive and anxiety states [3]. At the same time, loss as a trigger increases vulnerability to the manifestation of these disorders. Perhaps thus, there was no demonstrated significant difference in subjective feelings of distress by bereavement despite the history of loss.

Considering that we didn't find statistically significant differences in the clinical state of grieving for the person who died by complications of COVID-19, we thought that the availability and prevalence of coronavirus mortality information didn't add additional traumatic context. It is possible that in the context of the global crisis during the active period of spread and mortality, the fact of death due to complications of COVID-19 in most cases was perceived as an expected result. We recognize that people who have lost their loved ones due to complications of COVID-19 or other illnesses during this period may feel more vulnerable

and helpless in the context of anti-epidemic measures. At the same time, other factors, such as restrictions on access to traditional (religious) gatherings, social precautions against examples of irresponsibility in compliance with quarantine restrictions, and economic uncertainty, could significantly impact the process of prolonged grieving [6, 11, 13, 14].

Our results indicate that regardless of the period when lost loved ones, people who show signs of complicated grief have a more pronounced comorbid pathology, higher levels of psycho-emotional distress, and lower levels of satisfaction with the quality of their lives. At the same time, we noticed higher depressive symptoms among bereaved people during the pandemic.

The lack of statistically significant differences between other subgroups may indicate a general aspect of perceptions of distress and life challenges during a pandemic (Table III). We assume that quarantine measures themselves influenced the formation of distress and disrupted psycho-emotional aspects of adaptation to new living conditions. Still, they smoothed out the traumatic experience of bereavement as a sudden, unexpected crisis. In our study, results show significantly higher levels of psycho-emotional distress and the impact of traumatic experiences among people with signs of complicated grief who had suffered a bereavement before the pandemic than people without symptoms of complicated grief.

Also, the combination of the above factors may explain why people who do not show signs of complicated grief during the pandemic have more severe manifestations of depression and statistically significantly lower quality of life satisfaction than people who had suffered a bereavement before the anti-epidemic period.

As there was no statistically significant difference between the diagnosed disorders in the anamnesis, we cannot conclude whether the experience of widespread anxiety and affective disorders are independent vulnerabilities in the context of a pandemic situation. At the same time, the presence of anxiety and affective disorders in the anamnesis suggests that mastering strategies, typical beliefs, and behavioral strategies that are characteristic of these disorders may be modifying factors in the severity of comorbid pathology or influence the formation of complicated grief.

In summary, the analysis suggests that avoiding the social experience of post-loss interaction in the context of the pandemic can be seen as complementary to avoiding the traumatic experience of bereavement, limiting social contact through quarantine measures, and premorbid levels of social anxiety before assessment and the opinion of others about them or their status.

Despite the results, our study has several limitations. First of all, there are no valid diagnostic tools that meet the DSM-5 and ICD-11 criteria to diagnose signs of complicated grief. The ICG questionnaire is not validated in the languages spoken fluently in Ukraine. Therefore, the results of our study cannot be directly generalized as a diagnosis of complicated grief as an independent disorder, as they may be erroneous considering the cultural, religious, and ethnic aspects of the sample. The analysis was conducted on a Ukrainian-language sample without considering gender, ethnicity, or cultural (religious) traditions, which based on the analysis of vulnerabilities in a pandemic may affect the possibility of generalization. Second, although two experts verified the mental health assessment, we cannot say that the symptoms of other diseases not reported by the participants were mistakenly identified as signs of complicated grief. Also, expanding the sample and further analyzing and monitoring more than eight months after the bereavement could enrich our data and opportunities for interpretation.

CONCLUSIONS

The onset of the COVID-19 pandemic and the introduction of quarantine restrictions are essential factors affecting today's population's mental health and psychological well-being. At the same time, several social, economic, and cultural factors increase vulnerability to existing problems and increase general distress, thereby impairing our ability to adapt to new conditions. Even though a bereavement at any time is a significant shock, it becomes particularly relevant in the period of quarantine restrictions.

A person's ability to experience loss as a traumatic experience and grieve during the pandemic is modified with the context of the life situation and reflects those challenges that impose quarantine restrictions. In such circumstances, the psycho-emotional resource and the possibilities of resilience may be impaired. Automatic depressive beliefs about oneself, irrational anxious expectations and social avoidance, maladaptive behavioral strategies during the period of adaption to pandemic restrictions increase the prolongation of reactions to bereavement, the severity of comorbid pathology, and the risk of complicated grief.

Further research will expand and supplement the understanding of the mechanisms of the pandemic impact on the grieving process, which will help modify the recommendations and strategies of psychological care and support.

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ORIGINAL ARTICLE

NURSES' KNOWLEDGE ABOUT INFECTION CONTROL AT PRIMARY HEALTH CARE CENTERS IN AL-HILLA CITY, IRAQ

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ABSTRACT

The aim: To assess nurses' infection control knowledge at basic health care clinics and knowledge in practices nurses on infection control.

Materials and methods: This is cross-sectional study, conducted in primary health care centers within descriptive research in Al-Hilla City, from 17th May, 2021 to 2nd October, 2021. The researcher created the instruments to achieve the study's objectives through non-probability sampling: the purposive sample is made from of 140 searched, who worked in the dressing and immunization units of primary health care centers were chosen, and two study instruments [questionnaire and demographic data] were used for proper data collection.

Results: In this study revealed that (47.1%) at age groups (35-39) ages, the current study's findings revealed that (69.8%) of Males made up the sample and (60.7%) graduated from a nursing school (50%) have Training courses and (42.1%) consume (16-20) years of experience. And The nurses' general knowledge levels about infection control were good.

Conclusions: The majority of health-care personnel' knowledge, attitude, and practice regarding basic precautions were adequate, favorable, and safe by the expected standard.

KEY WORDS: Nurses, Infection Control, Knowledge

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INTRODUCTION

The Alma-Ata Declaration defines primary health care (PHC) as "essential health care based on practical, scientifically sound, and socially acceptable methods and technology made universally accessible to individuals and families in the community through their full participation at a cost that the community and country can afford to maintain at every stage of their development in the spirit of self-reliance and self-determination at a cost that the community and country can afford to maintain at a cost that the community and country can afford to maintain at a cost [1]. Used standard precautions by healthcare personnel has been identified as an effective method of preventing and controlling healthcare-associated illnesses. These precautions safeguard not just the patient, but also the medical personnel and the environment [2]. Infectious diseases are caused by pathogenic microbes such as bacteria, viruses, parasites, and fungus. The diseases can be passed from one person to another, either directly or indirectly, through fluid exchange or exposure to vectors, or through the environment [3]. Infections acquired while receiving medical care result in death, disability, and increased medical expenses. In the absence of infrastructure to properly implement new technology, there is a risk of negative consequences. As a result, in the primary health maintenance facility, you should take a comprehensive approach to the prevention and control of infections [4]. Infection control in health-care settings reduces the incidence of nosocomial infections, which reduces morbidity and mortality. It's also connected to a shorter stay in the hospital, ranging from one day to several weeks. As a result, the purpose of the research was to determine how effective infection control recommendations are in practice among health-care personnel [5]. The importance of a good health system in promoting maternal health and minimizing maternal mortality is widely recognized. To achieve favorable health outcomes, effective maternity coverage involves timely and affordable access to appropriate, high-quality, and safe care for all segments of the population. All health systems try to increase access, safety, and quality, and these factors are especially crucial when it comes to infections caused by childbirth. A well-functioning health system is required to improve and maintain infection control as part of delivery care [6]. Infection control and staffing are less expensive as compared to the costs of treating patients or treating nurse-acquired infections. As a result, health-care workers should be bound by professional and ethical requirements to keep their infection-control knowledge and skills up to date, and to perform safely and competently at all times [7]. Infection prevention begins with early identification and surveillance measures, and health-care professionals play a critical role. As a result, substantial training is essential to ensure that these principles are understood and implemented in all health-care settings. Because the quality of nursing care is largely determined by the knowledge, skills, attitudes, and behaviors of practicing health care employees, health care personnel must be educated in

Table I. The spreading of study tasters by demographic characteristic

Demographic Characteristic			Demographic Characteristic		
age (year)	**F**	**%**	**Level of education**	**F**	**%**
20 -24	11	7.8	Secondary	38	27.2
25 -29	16	11.5	Institute	85	60.7
30 -34	38	27.2	College and above	17	12.1
35-39	66	47.1	Total	140	100%
40-44	6	4.3	Turning course	F.	%
45 more than	3	2.1	None	23	16.3
Total	140	100.0	1-3 years	32	20.8
Mean =24.64S.D = 0.28			4-6 years	18	12.9
Residency	F.	%	7 more than	72	50
urban	84	60.	Total	140	100.0
Outskirts	36	25.7	Years of Employment	F	%
rural	20	14.3	1-5 years	14	10
Total	140	100.0	6-10 years	24	17.1
Gender	F.	%	11-15 years	13	9.3
Male	92	69.8	16-20years	59	42.1
Female	48	30.2	21 more than	30	21.4
Total	220	100.0	Total	140	100%

basic infection control concepts and acquire new knowledge and skills [8]. Goal from this study is determined the rate of cesarean section and to describe the contributing factors of (CS) among pregnant women admitted to maternity hospitals in Babylon province, because higher numbers of cesarean sections are associated with increased risks for the mother and her newborn, and may complicate future pregnancies, as well as a high burden on the health system's resources [9].

THE AIM

The aim was to assess nurses' infection control knowledge at basic health care clinics and knowledge in practices nurses on infection control.

MATERIALS AND METHODS

This is cross-sectional n primary health care centers, a graphic research was conducted in Al-Hilla City, from 17th May, 2021 to 2nd October, 2021. The researcher created the instruments to achieve the study's goals using "non-probability sampling: a purposive sample of" 140 nurses who worked in the dressing and immunization units at primary health care centers was chosen, and two study instruments [questionnaire and demographic information] were used for proper data collection. The instrument's reliability was confirmed by "test and retest', and the instrument's validity was determined by a panel of specialists.

ETHICAL APPROVAL

Information was provided by Kut Community University. ethics committee, as well as the consent of the sectors management. After explaining the study's objectives and data requirements to them, all participants who accepted to participate in the current investigation gave their written consent. Data was handled with caution, and certain measures were implemented to ensure that the study's data was obtained in a secure manner. This study's statistical analysis was carried out using SPSS Software version 26. Continuous numerical descriptive statistics (frequency, "percentages, arithmetic mean, and standard deviation) and arithmeticalillative were employed to analyze the data (Relative Sufficiency).

RESULTS AND DISCUSSION

This study involved a total of 140 revealed that 47.1% at age groups 35-39 ages, Males made up 69.8% of the sample, according to the findings of the current study, 60.7% are graduates of a nursing school, 50% training courses and 42.1% have 16-20 years of experience.

Table II shows that highdomains relative to the nurse's knowledge about infection control regarding the overall nurses' knowledge are high.

Table III shows that high items relative to the levels of nurse's knowledge about infection control are good.

The study's findings are thoroughly addressed, with supporting evidence presented where accessible in the literature and in relation to the study's aims table 1 shows the results of the current investigation, which revealed that 47.1% of the age groups 35-39 years. This figure is consistent with the amount of respondent'slong-standing reported in a research, which found the majority of their study

Table II. Nurses Knowledge about Infection Control

No.	Domains	M.S	R. S	Grade
1	A General Knowledge	2.55	85	High
2	Infection's Reasons	2.44	81.3	High
3	Anti-Infection Measures	2.46	82	High
4	Sterilization and Disinfection	2.66	88.6	High
5	Management of Waste	2.48	82.6	High
Overall Nurses' Knowledge		2.51	83.6	High

Table III. Nurses' Knowledge Levels

No.	Levels	Frequency	Percentage
1	Poor	16	11.5%
2	Fair	36	25.5%
3	Good	88	63%
4	Total	140	100%

sample 55%, 24.7% is made up of nurses. Furthermore, this conclusion is consistent with the findings of a study done by [10]. According to a study, less than half of nurses are above the age of 50(45.5%). Males account for more than half of the participants in the study 69.8%. This result is in line with [11]. The majority of the study's response are men, according to the findings 69.7%. The majority of the participants in this study were nursing institute graduates, according to the data 60.7%. These conclusions have been backed up by research [12] who have revealed that more of the participants were diploma 47.33%. In addition, I decide with the findings of a research done through [13]. Diploma holders were among the attendees 46.33%. In addition, I approve with the verdicts of a study done by [14], more than 42.1% of nurses with a diploma have 16-20 years of experience, according to the majority 93.2%. These conclusions have been backed up by research [15] who stated that more than half of the participants 30% have worked for 1 to 5 years. According to our research, more than half of the participants 50% attended training sessions. The findings of this study are similar to those of earlier studies undertaken by the author [16], 60% of them had fewer than five years of experience and 60% had completed an infection control training session, according to the researcher. The findings are consistent with nursing literature.According to the study's findings, there are a lot of domains. to the nurse's knowledge about infection control regarding the overall nurses' knowledge are high [17] study indicated the training to improve nurse's knowledge concerning infectious diseases. She said that "There were significant statistical differences in nurse'sknowledge, practices, and attitudes after training of the infectious diseases control with their knowledge, practices, and attitudes in period of prior training courses.

CONCLUSIONS

The majority of health-care personnel' knowledge, attitude, and practice regarding basic precautions were adequate, favorable, and safe by the expected standard. By providing training and exposing health care personnel to infection, we can strengthen and integrate universal precaution with everyday services.

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ORIGINAL ARTICLE

THE ISSUE OF HISTOLOGICAL IDENTIFICATION OF M-CELLS IN THE PEYER'S PATCHES OF ALBINO RAT SMALL INTESTINE

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ABSTRACT

The aim: Based on the above cytological signs of M-cells, we set the goal of more detailed clarification of some of their topological relationships with other enterocytes in the follicle-associated epithelium of Peyer's patches of albino rat small intestine.

Materials and methods: 10 mature albino male rats weighted 200,0±20,0 g were involved into the study. Anatomical dissection with the sampling of the sections of the small intestine containing Peyer's patches was carried out with subsequent embedment of the latter into paraffin blocks and making of serial histological sections of 4 μm thick in the cross-section of the small intestine, followed with hematoxylin-eosin staining. The specimens were studied and documented on the "Konus" light microscope equipped. Morphometric characteristics of the specimen tissue structures were studied using the Sigeta X 1 mm/100 Div.x0.01mm stage micrometer.

Results: The findings of the study revealed enterocytes with phagocytic properties found in the lymphoid-associated epithelium of Peyer's patches of the small intestine of albino rats. Moreover, if they are clearly visualized at the light-optical level, then M-cells are poorly recognizable, which is consistent with a similar assessment made by other authors.

Conclusions: Given this, the issue on the topology and functional purpose of M-cells remains uncertain to date and, thereby, the prospect of further research is being outlined, which, in our opinion, can be successful using the method of stereomorphological analysis. For this purpose, multilayer plastic reconstruction methods can be used for serial semi-thin sections of Peyer's patches embedded in epoxy resin, according to the requirements of transmission electron microscopy.

KEY WORDS: lymphoid-associated epithelium, Peyer's patches, small intestine, albino rats, M-cells, phagocytic enterocytes

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INTRODUCTION

It is generally accepted that initiation of the intestine immune responses is triggered by the specialized cells within enterocytes, overlying the luminal surface of the Peyer's patches. They are commonly known as M-cells, which, unlike typical enterocytes (according to popular opinion) have a reduced cytoplasm due to the presence of a basolateral pocket, where lymphocytes, macrophages and dendritic cells are populated [1-4].

M-cells are often called immunosurveillance posts in the intestinal epithelium. Publications report that in the epithelium, overlying the Peyer's patches (follicle-associated epithelium), they account for no more than 10% [5, 6]. It is believed that due to their transcytotic activity, M-cells are able to transfer various antigens from the intestinal contents into the basolateral pocket unchanged, where they are captured by dendritic cells and macrophages that, as a result of processing, present the antigen to T-lymphocytes, thereby launching the development of immune responses [3, 5, 7-9].

THE AIM

Based on the above cytological signs of M-cells, we set the goal of more detailed clarification of some of their topological relationships with other enterocytes in the follicle-associated epithelium of Peyer's patches of albino rat small intestine.

MATERIALS AND METHODS

10 mature albino male rats weighted 200,0±20,0 g were involved into the study. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) at the Ukrainian Medical Stomatological Academy in compliance with the regulations on keeping experimental animals, adopted by the European Parliament and Council Directive (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013), (Minutes No. 178 as of 24.12.2019 of meeting the Commission on Biomedical Ethics at Ukrainian Medical Stomatological Academy) [10-12].

After vivisection made by thiopental anesthesia overdose (75 mg/kg of animal body weight intramuscularly in the upper third of the thigh of the hind paw) [13] in

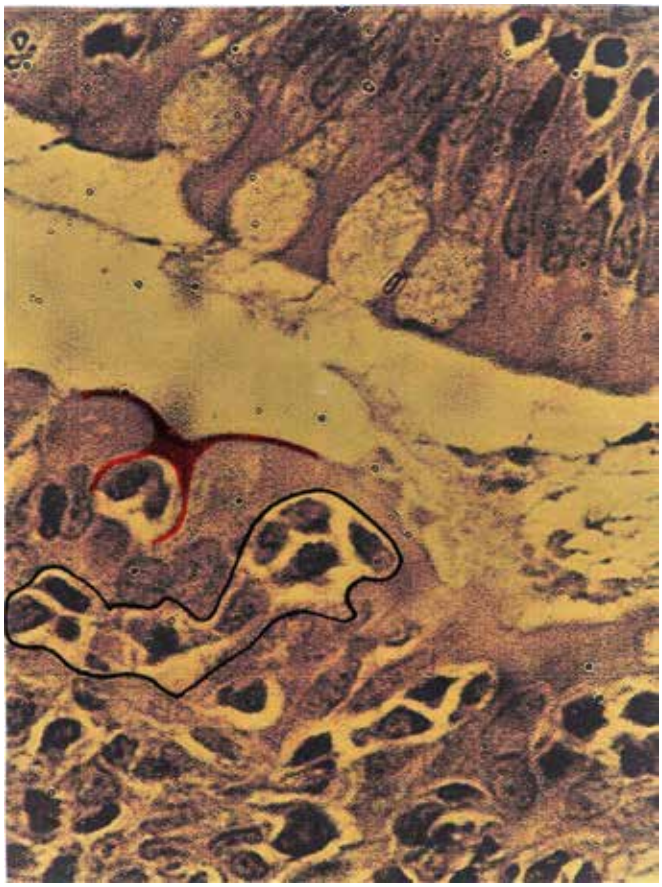

Fig. 1. Microscopic structure of the segment of lymphoid-associated epithelium of the big lymphoid nodule and adjacent intestinal villus of the Peyer's patch of the small intestine of albino rats. The black outline indicates the border of the intraepithelial lymphoid compartment, and the putative M-cell is colored red. Paraffin section; H&E stain; 100× magnification.

compliance with the requirements for dissection of the abdominal cavity, the entire complex of the gastrointestinal tract was removed, which was fixed in 10% formalin solution for two days. Following a week (after pre-washing in a running water) the murine gastrointestinal tract was examined and sections of the small intestine, containing Peyer's patches, were selectively excised. The set of Peyer's patches were clearly visualized along its length beneath the serous membrane (on the side opposite to the mesentery attachment site) in the form of whitish spots, different in shape and size.

The specimens, after washing from formalin and dehydration in alcohol of increasing concentration, were embedded into paraffin blocks, from which serial sections of 4 μm thick (Microm HM 325) were obtained in the cross-section of the small intestine and, subsequently, stained with hematoxylin-eosin. Their study and documentation was carried out using the "Konus" light microscope equipped with the Sigeta DCM-900 9.0MP digital microphoto attachment and the Biorex 3 program (serial number 5604) adapted for these studies. The morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system of visual analysis

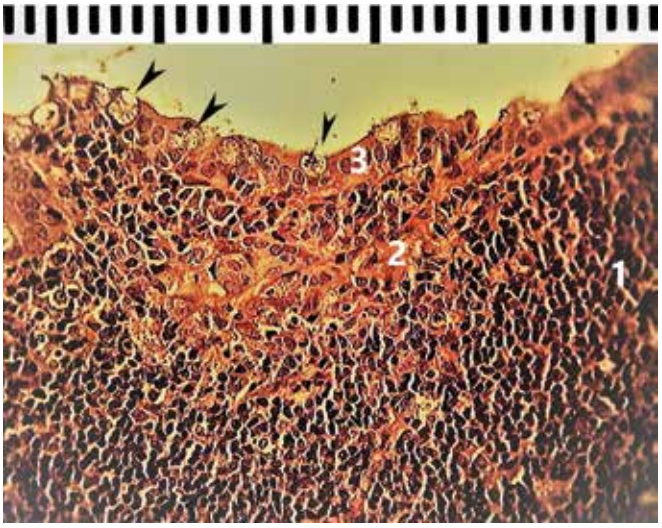

Fig. 2. The apical part of the lymphoid nodule of Peyer's patch of the small intestine. Paraffin section; H&E stain; 40× magnification (the step of the scale is equal 10 μm).
1 – lymphocytic elements; 2 – connective tissue layers; 3 – enterocytes with phagocytotic phenomena within lymphoid-associated epithelium (marked by arrows).

of histological specimens, as well as using the Sigeta X 1 mm/100 Div.x0.01mm stage micrometer, the scale of which (equal to 1 mm, where a small step corresponds to 10 μm) was applied to the corresponding microimage obtained in the same magnification.

RESULTS

A thorough targeted study of serial paraffin sections of Peyer's patches revealed an extremely large variety of configurational features of their epithelial overlay, which is called the follicle-associated epithelium in the literature. Noteworthy, such a name cannot be considered correct, because the word "follicle" used in it is an anachronism in relation to the formations that make up Peyer's patches. It is known, they are called lymphoid nodules. Therefore, the epithelium, overlaying them, is more correctly called the "lymphoid-associated" epithelium, which we will use hereinafter [14-18]. In our opinion, this configurational variability depends on situationally changing factors of antigenic effect, i.e., functional polymorphism is characteristic of lymphoid-associated epithelium of lymphoid nodules of Peyer's patches, usually not highlighted in the literature.

But most often, it clearly reveals the cluster pattern of distribution of enterocytes in the form of epithelial buds, which were described in the previous publications. It has been established that some of these cluster groups have the form of specific compartments, the inner contents of which are represented by small grouped aggregations of lymphocytic elements separated from the intestinal contents by a thinned layer of enterocytes (Fig. 1). At the same time, with great difficulty (as a result of multiple viewing of serial sections), it was possible to identify specifically shaped cellular elements within superficially

thinned epithelium, which are characterized by the presence of extended cytoplasmic processes, circumferencing a relatively extensive lymphoepithelial compartment. We believe that it is the hardly recognizable cellular elements that can be assigned to M-cells, since it was not possible to identify other cells corresponding to those described in the literature as part of the lymphoid-associated epithelium of Peyer's patches of the small intestine of albino rats. Of note, according to most authors, the ultimate cytological sign of M-cells is the presence of cytoplasmic invagination called a "pocket" or "niche", resided by lymphocytes together with dendritic cells and macrophages, in their basolateral part [4, 19, 20]. Consequently, the question arises: can an epithelial cell with a size of only about 15 μm have invagination of such a depth that would accommodate at least several lymphocytes, including macrophages and dendritic cells? Apparently, the answer to this question is obvious. And it should be noted that the authors, who kept to this opinion, present as an evidence arbitrary corresponding graphic drawings only, but not microphotographs, and if the latter are given, then these elements noted as M-cells are not indisputable [21].

DISCUSSION

The fact is that the lymphoid-associated epithelium of Peyer's patches is a polarized monolayer populated by various types of enterocytes, including predominant absorbing cells, as well as endocrinocytes, goblet cells and tuft cells, which are visualized mainly by electron microscopy [22-24]. Importantly, cytological differentiation of lymphoid-associated epithelium is complicated by the fact that some authors are inclined to believe that M-cells and tuft cells are the same [25].

Notably, the study revealed clear signs of plasmolemma rupture in the apical part of some enterocytes of Peyer's patches and the presence of a granular conglomerate of unknown nature (Fig. 2). This phenomenon can be interpreted in two ways: either this refers to the moment of extrusion of secretion products, for example, from goblet cells, or the process of phagocytosis by a cell of some parietal material is observed. According to our data, this phenomenon is observed in alternating order over almost the entire surface of the lymphoid-associated epithelium. If we assume the process of phagocytosis, then the question arises as to the type of cells it is carried out. By all cytological features, they cannot be identified with M-cells, which, according to existing ideas, possess such properties.

To clarify this, publications report that almost all enterocytes of lymphoid-associated epithelium are capable of phagocytosis, including goblet cells [26-28]. It is obvious that all this does not fully fit into the concept of M-cells as the only cellular structures that mediate between antigens of intestinal contents and lymphoid tissue of Peyer's patches.

CONCLUSIONS

Thus, the findings of the study revealed enterocytes with phagocytic properties found in the lymphoid-associated epithelium of Peyer's patches of the small intestine of albino rats. Moreover, if they are clearly visualized at the light-optical level, then M-cells are poorly recognizable, which is consistent with a similar assessment made by other authors.

Given this, the issue on the topology and functional purpose of M-cells remains uncertain to date and, thereby, the prospect of further research is being outlined, which, in our opinion, can be successful using the method of stereomorphological analysis. For this purpose, multilayer plastic reconstruction methods can be used for serial semi-thin sections of Peyer's patches embedded in epoxy resin, according to the requirements of transmission electron microscopy.

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ORIGINAL ARTICLE

PHYSIOLOGICAL AND HISTOLOGICAL STUDY OF THE CALCIUM OXIDE NANOPARTICLES EFFECT ON THE TESTIS OF MALE WISTER RATS

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ABSTRACT

The aim: The study carried out to determine the effect of the Calcium Oxide Nano Particles on the male rats. In this study were used calcium oxide nano powder to enhancing apoptosis in germ cells and disrupting hormonal regulation of reproductive processes in the adult male rats.

Materials and methods: The experiment using nine male rats, were distributed into three groups. Group one A was doses orally of Calcium Oxide Nano Particles 50 mg/kg of body weight, while group two B was doses orally of Calcium Oxide Nano Particles 100 mg/kg of body weight; also, group three C were a control C group treated with 0.9% saline only, these orally doses continuous 10 days. After 10 days blood samples collected and all rats were euthanatized and the weights, histological changes and hormonal analysis were conducted.

Results: Results show a significantly increased at $p<0.05$ for both groups A & B compare with control C in weight of testis tissue and the level of testosterone hormone, also histology changes in testis of treatment rats include: necrosis of spermatogonia, primary spermoocyte and spermatids in both groups compared to control group.

Conclusions: Through the study, it is recommended to use medium or low doses of CaO NPs that can be used as a testicular tonic and urge it to increase production of the Testosterone hormone**.**

KEY WORDS: Nanomaterials, Calcium oxide**,** Male rats**,** Testosterone**,** Hormone**,** Spermatogonia, Somniferous

Wiad Lek. 2022;75(5 p2):1313-1316

INTRODUCTION

The nanomaterial's industry has entered most of the materials it consumes or uses for humans, whether in its food, clothing or various other applications, as nanomaterial have entered the generally in medical field and particularly in dentistry. Some fillings made of nanomaterial may be used or may be enter the dental industry. As nanomaterials, can used in agriculture, engineering and other applications. In vitro studies have been increasing constantly; in vivo studies of nanoparticles have not established a unified system until now. It is well known that a male reproductive system is more sensitive and vulnerable to various stresses such as heavy metals, xenobiotic compounds, microwaves, and nanoparticles than other organ systems [1-3]. Some studies have shown the negative effects of some NPs such as Ag on reproductive indicators such as increasing abnormal sperm, altering testes structure, decreasing the expression of spermatogenesis genes, lowering serum testosterne, enhancing apoptosis in germ cells and disrupting hormonal regulation of reproductive processes in the male rats [4-10]. Accordingly, a study carried out to determine the effect of these substances on the male rats. There was a necessary to verify the exposures of animals and humans to more one than types of nanoparticles and entry routes for environmental, therapeutic, diagnostic, and cosmetic uses. These NP such as Au, TiO2 may transfer to fetus, with varying toxic effect on different organs such as brain, kidney, liver, and reproductive system [12-13]. Calcium oxide nanoparticles have attention due to compatibility [13], calcium oxide – nanoparticles may lead to toxic effect in male rats [14]. In the current study, the toxicity can cause morphological changes in testis of male rats.

THE AIM

The study carried out to determine the effect of the Calcium Oxide Nano Particles on the male rats. In this study were used calcium oxide nano powder to enhancing apoptosis in germ cells and disrupting hormonal regulation of reproductive processes in the adult male rats.

MATERIALS AND METHODS

Calcium oxide Nano powder purchased from Iraqi Nanomaterials Pioneers Company, (Najaf, Iraq). The CaO nanoparticles with a desired amount (0.0075g) was placed in 30 ml of re-distilled water and was sonicated for 5 h to separate CaO nanoparticles and overcome the

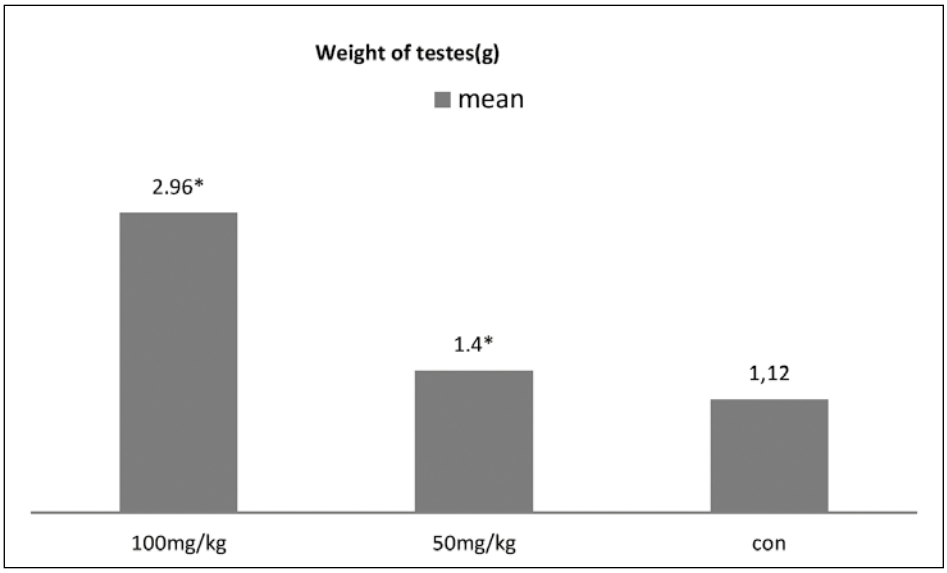

Fig. 1. The Testis weights (g) in male rats of studied groups compared with control group

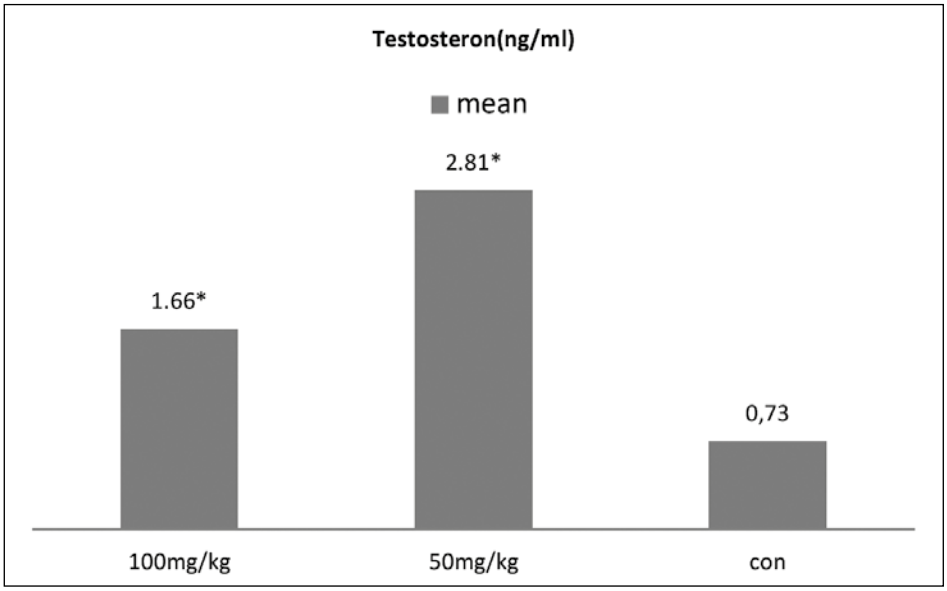

Fig. 2. The Testosterone hormone (ng/ml) in male rats of studied groups compared with control group

agglomeration and make the colloidal contain individual nanoparticles. The Formulation of Nanoparticle Suspension: the suspending CaO prepared a stock solution of the suspension -nanoparticles in the bi- distilled water. The experiment using nine male rats, animals acquired from University of Karbala, and then housed in clean cages in Science Faculty laboratory. Distilled water (D.W.), and trade food bits for rats were obtainable. After first week acclimation, the suspension of CaO-NPs given to treated animal male rats for 10 days daily. Animals were randomly divided into three groups, each group including three rats: Control group(C) treated with 0.9% saline only and the other groups (A&B) treated with CaO-NPs suspension (50 and 100 mg/kg) from body weight managed orally for 10 days. On the 11th day, blood was collected from heart and the serum separated by the centrifuge. After collecting blood samples testosterone hormones was measured by an automatic Fujifilm (Dry chemN×500), made in Japan (2014) and Mini Vidas (2000). All rats euthanatized and testis obtained for histological study. The testis were weighting and then placed in ten percent from buffered formalin for (24) hours, then dehydration, clearing, infiltration, embedded in paraffin, sectioning, stained with hematoxylin and eosin. Data were analyzed statistically SPSS/version 19 (Mean ± Standard deviation), significance considered at p value less than 0.05.

RESULTS

The study shows as appear in figures (1) and (2) significantly increased in testis weights and testosterone hormone in both experimental groups as compare with control group. From figure (1), it is noted that the level of the male hormone testosterone is (50 mg/kg) using the dose.

Figure (2) refereed that the high concentration (100 mg/kg) used in the second dose led to a complete destruction of the testicular tubes, and thus a significant decrease in the level of male hormone observed in the rats that given

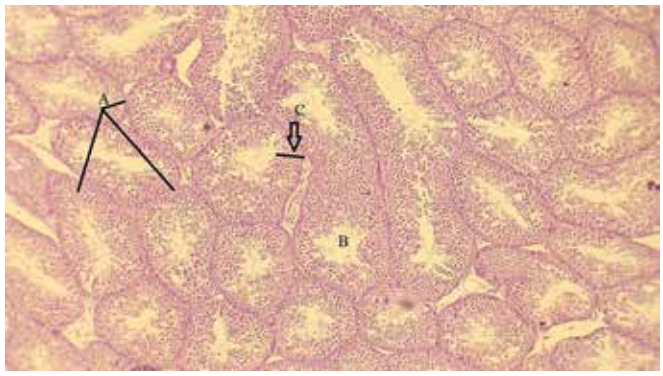

Fig. 3. Transverse section of seminiferous tubule of control rats (H&E 10x), A- seminiferous tubules, B-lumen of seminiferous tubules, C- deferent stages of normal spermatogenesis

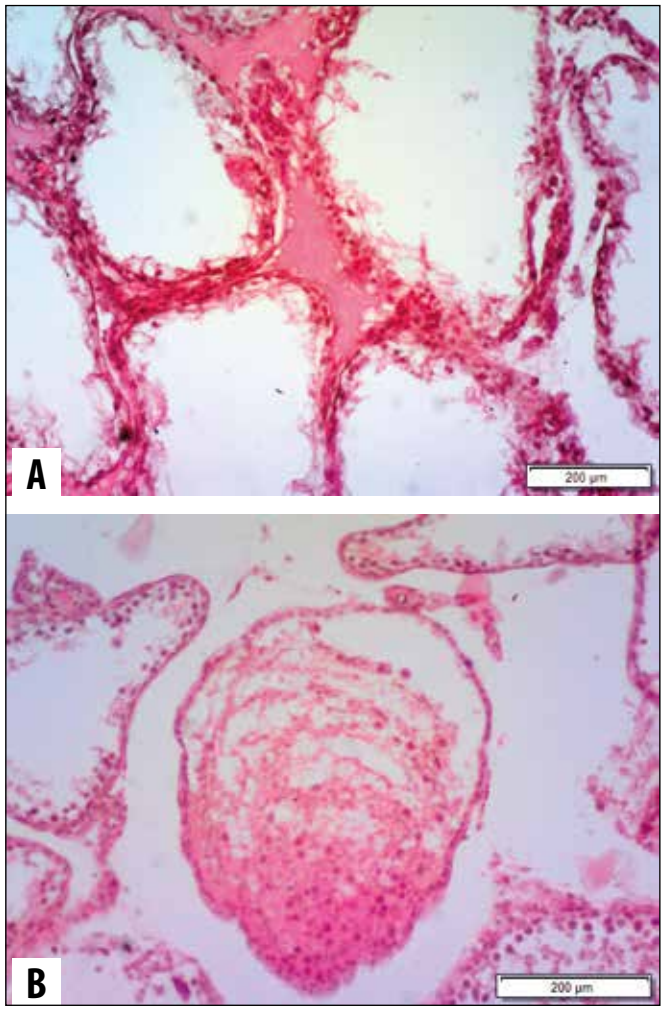

Fig. 4. (A): Section in Photomicrograph of testes of treated rats.F: Edematous fluid, N: Necrosis, Stain(H&E), A: X100. (B): Section in Photomicrograph of testes of treated rats.N: Necrosis, Stain (H&E), B: X100. Enlargement of inter seminiferous tubules spaces figure (4A).

the high concentration, which confirms the advanced results, fig 1 and fig 4B where it is noted that there is a significant weight gain in the testes of rats that were given high concentration (100 mg/kg). This explained also by figure (2)

Figure 3 shows the normal histological structure of the testis in the male rats used in the study, showing the testicular tubes and all showing the different stages of sperm formation (spermatogenesis). All of Histopathological changes appeared in (fig4B), figure (3A) and (fig4 A&B).

DISCUSSION

In this study may be enlargement of inter seminiferous tubules and edematous exudate fluid. These results accepted with other studies that say main problems are declined spermatozoon count and inhibition testosterone hormone [14-15].

Through the study, it is recommended to use medium or low doses that can be used as a testicular tonic and urge it to increase production of this hormone, as such a result was not referred to in research related to studying the effect of Nanocomposites on such results.

In addition, high concentration of dose leads to increase weight gain in the testes caused by swelling (edema) in the testicular tissue, as well as what has been confirmed by where the noticeable decrease in the level of the male hormone testosterone appears.

Histopathological examination of the treatment group with Calcium Oxide Nanoparticles showed Necrosis of spermatogonia, primary spermatocytes and spermatids compared with control group, where seminiferous tubules lumen. Enlargement of inter seminiferous tubules spaces with penetration of Edematous exudate fluid. In addition, debris of necrotic cells observed in seminiferous tubules lumen, empty seminiferous tubules lumen due to necrosis of male germs cells and Sertoli cells also observed compared with control group in figures above.

Studies showed the toxicity can cause histological changes intestis of male rats, agree with [16] was reported that the calcium oxide- nanoparticles in rats can causes toxicity.

Other study agrees with a report with different Nanoparticles [17]. Main complications are declined spermatozoon count, also degenerative in tissue of testis and reduced number of spermatozoon in seminiferous tubules is obvious in poisoning in male rabbits, this study agrees with results in [18].

CONCLUSIONS

Through the study, it is recommended to use medium or low doses of CaO NPs that can be used as a testicular tonic and urge it to increase production of The Testosterone hormone, but the using a high doses may be causes declined spermatozoon count and decrease in the level of male hormone in male rats.

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ORIGINAL ARTICLE

THE PROGNOSTIC UTILITY OF LEUKOCYTE AND PLATELET COUNTS FOR RISK ASSESSMENT OF IN-HOSPITAL COMPLICATIONS IN PATIENTS WITH ACUTE ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION

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ABSTRACT

The aim: We have aimed to develop method of risk assessment in patients with acute STEMI using blood cells count useful in everyday clinical practice.

Materials and methods: 317 STEMI patients admitted to ICCU starting at 01/2014 to 06/2020 were included in this study. Data were evaluated partially retrospectively (n=214), 1st group) and prospectively, applying new risk-assessment tool in 103 patients 2nd group). Study groups did not differ in terms of all clinical characteristics.

Results: To evaluate criteria of severity of in-hospital complication we have created in-hospital complication index (HCI), composed according to number of adverse events per patient. Based on correlation analyses we have composed complex WBC-platelets (WBC-PLT) index = ((GRA – MON) / LYM) · 10 + PDWc + P-LCR). In the 1st study group WBC-PLT index was associated with in-hospital complications' index and correlated with neutrophil-leukocytes ratio (NLR) ($p<0,001$ and $p<0,0005$ respectively). WBC-PLT index > 137 appeared to be mildly associated with increased risk of adverse in-hospital outcomes (with specificity of 78 % and low sensitivity of 64 %, AUC- 0,72). Further prospective study of WBC-PLT index measured on the first STEMI-day (group 2) revealed that this parameter was more informative regarding association with adverse in-hospital events whereas NLR had low accuracy in risk assessment.

Conclusions: WBC-PLT index assessed on the first STEMI day may be used as a tool for detection of patients at risk of adverse in-hospital events in the every-day clinical practice.

KEY WORDS: STEMI, blood cells count, risk evaluation, WBC-platelet index

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INTRODUCTION

Cardiovascular diseases are the leading cause of death worldwide [1, 2]. This primarily applies to morbidity, complications and mortality of acute coronary syndrome (ACS). Therefore, it is important to search for markers that may accurate and easy identify patients at risk of adverse outcomes of ACS [3-6].

Congenital and adaptive immune pathways play an important role in the development and progression of many cardiovascular diseases [7]. Activation of systemic inflammation underlies may trigger plaque destabilization and is closely related to neutrophils and monocytes - components of innate immunity. Neutrophilic granulocytes are involved into the initial phase of pro-inflammatory processes activation accompanying tissue damage, while activated monocytes and macrophages support inflammation at the site of injury and participate in its subsequent healing [7-10].

Nowadays C-reactive protein (CRP) and interleukin-6 (IL-6) along with other parameters e.g. white blood cells (WBC), erythrocyte sedimentation rate (ESR) and ratios of individual blood cell populations are easily accessible in everyday clinical practice. [11].

Under conditions of atherosclerotic plaque rupture and on-going thrombosis leukocyte-platelet defense mechanisms may accelerate atherothrombosis, leading to further myocardial damage and resistance to antithrombotic therapy. [12].

Neutrophil - lymphocyte ratio (NLR) is reported to be a marker of adverse cardiovascular events in patients with STE ACS. [11]. Neutrophils have mostly pro-inflammatory properties, which are regulated by lymphocytes [6].

Neutrophils, monocytes and their ratio may be considered as potential surrogate systemic inflammatory response markers and also correlate with CRP levels which is known to be associated with clinical course of cardiovascular disease [13-15].

Nowadays variety of scores and scales encompass risk assessment in patients with ACS and contain clinical and laboratory indicators. However, they require external validation accounting for accessibility of laboratory data in local health care [11].

THE AIM

The aim of the work is to create a new method for assessing the risk of complications in STEMI patients using simple tool as blood cell counts and access it's practical value.

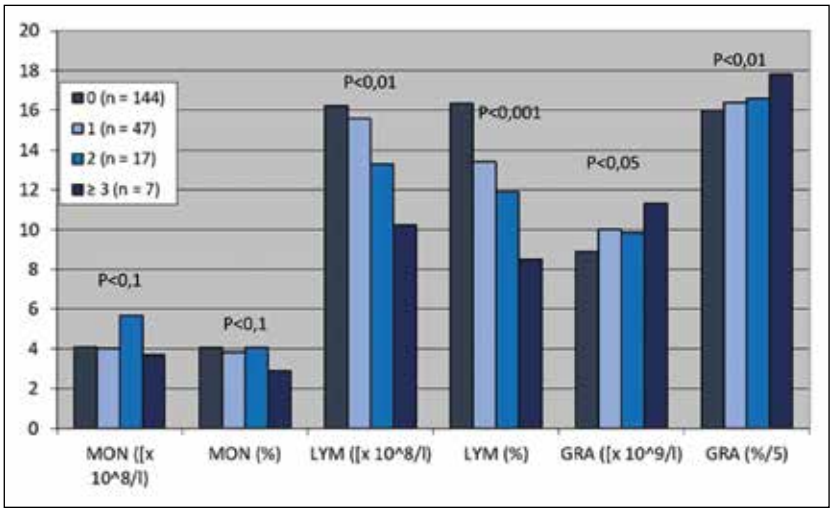

Fig. 1. Values of WBC counts on 1st STEMI day in patients with one, two, three or more in-hospital complication of STEMI.

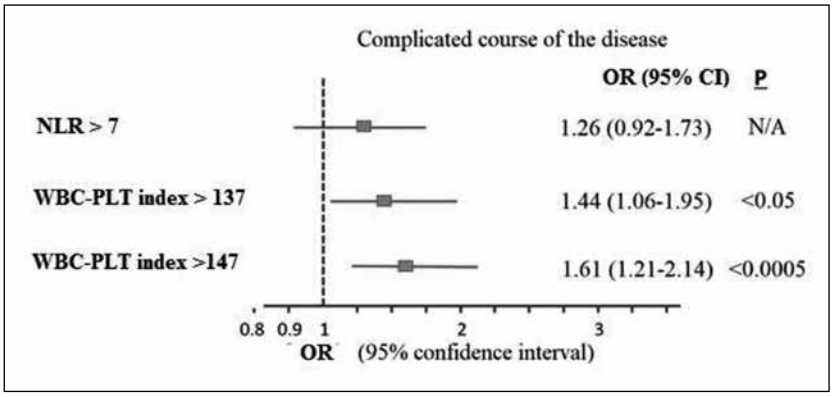

Fig. 2. Prospective assessment of association between laboratory indices (1st STEMI day) and development of in-hospital STEMI complications (n=103).

MATERIALS AND METHODS

We have enrolled 317 STEMI patients who were hospitalized from 01/2014 to 06/2020 to ICCU at Ukrainian Institute of Cardiology during first 12 hours after symptoms onset. STEMI was diagnosed using clinical, electrocardiograchic and laboratory criteria according to current ESC Guidelines and Guidelines of Ukrainian Association of Cardiology [16, 17]. Data of 214 patients (1st group) were studies retrospectively and developed risk-assessment tool of adverse in-hospital outcomes was validated prospectively in 103 patients (2nd group).

Criteria of in-hospital course severity were acute LV failure (Killip II-III), recurrent MI, early post-infarction angina, presence of acute left ventricular aneurysm, floating LV thrombi, onset of atrial fibrillation with haemodynamic compromise. These criteria were included in in-hospital complications score ranging from 0 to 5 points. Patients with cardiogenic shock severe kidney and/or liver dysfunction, acute inflammatory disease, anemia and stroke were excluded from the study.

Informed consent to participate in this study was obtained from all patients.

Patients received standard therapy of STEMI according to current ESC guidelines. In addition to this majority of patients also received metabolic therapy (trimetasidine, soluble quercetine).

All investigations were performed on the 1st and 8th days of STEMI and included echocardiography (using SSH-880CV Aplio Artida, Toshiba Medical Systems Corporation, Japan), blood cells counts with basic characteristics of the main types of cells (using hematologic system «Elite 3», Chech Republic), blood biochemistry («A-25» system and reagents BioSystems, Spain), serum CRP (semi-automatic BTS-350 system, Spain).

Statistical analyses were performed using applying Microsoft Excel 2016, Statistica (StatSoft Inc, 7.0.61.0) and SPSS 17.0 software. Descriptive statistics, tests for normality of distribution for continuous data, ANOVA, non-parametric Mann-Whitney, Wilcoxon tests, Friedman's tests, Pearson's and Spearman'correlations, ROC were applied.

RESULTS

Patients of 2nd group were older and have less history of MI but these differences between 1st and 2nd study groups did not reach statistical significance.

In the 1st patients' group (n=214) we have studied correlations between laboratory markers of adverse clinical course of AMI (monocytes, WBC, CRP (n = 63), glomerular filtration rate (GFR), glucose, fibrinogen, mean platelet volume (MPV), platelet distribution width (PDWc), % of large (– > 12 fL) – platelets platelet large cell ratio (P-LCR), low density lipoproteins (LDL) cholesterol) and in-hospital complications index (HCI) (Table II).

We have noted significant reverse association between HCI and lymphocytes proportion (p<0,001). Number of in-hospital complications were straightly associated to

Table I. Characteristics of STEMI patients

	1st group (n=214)	2nd group (n=103)
Age, years	59,0±0,8	64,4±1,1
Male sex, %	78,09	72,8
Hypertension, %	73,8	77,7
Diabetes mellitus, %	13,6	22,3
BMI > 30 kg/m^2, %	25,7	24,3
Prior MI history, %	13,6	5,8
History of revascularization, %	0,9	2
Stroke history, %	5,6	4,9
Atrial fibrillation, %	5,6	13,6

Table II. Correlations between in-hospital complications index, blood cells counts and laboratory risk indicators of adverse clinical course of acute MI

Indicator	Correlation between HCI and risk indicator values (rh0)
MON, ·10^9/l	–0,12
MON, %	–0,13
LYM, ·10^9/l	–0,18**
LYM, %	–0,26***
GRA, ·10^9/l	0,16*
GRA, %	0,21**
PDWc	0,15*
P-LCR	0,20**
MPV	0,09
WBC	0,19**
CRP (n = 63)	0,14
GFR (CKD-EPI)	–0,12
Glucose	0,09
C-LDL	–0,09
Fibrinogen	0,06

Notes: Statistical significance of association: * p<0,05; ** p<0,01; *** p<0,001

granulocytes counts to less extent and did not have any correlation to monocytes counts. These data may explain weak association between WBC counts and HCI. PDWc and P-LCR were also associated with HCI.

Further we have analyzed association between in-hospital complications count in the 1st group of patients and leucocytes subtypes counts on the first STEMI day. Fugure 1 reflects obtained data.

Table III. Assessment of association between WBC counts, CRP levels and PDWc on the 1st STEMI day

Variable	CRP (n = 63)	PDWc	P-LCR
MON	0,29*	0,15	0,07
LYM	–0,06	–0,02	–0,02
GRA	0,01	0,10	0,09

Note:* - Statistical significance p<0,05.

Table IV. Correlations between STEMI in-hospital complications number and baseline laboratory data derived indices (Spearman's correlation)

Parameter	Correlation with HCO (rho)
WBC-PLT-CRP index	0,34***
WBC-PLT index	0,33**
NLR	0,22*
PLR	0,19*

Statistical significance: * p<0,01; ** p<0,001; *** P<0,0005.

Lymphocytes and their proportion were linearly related to the number of hospital complications. (Fig. 1). Patients with greater number of complication had also greater granulocytes counts, more pronounced in patients with three or more complications, Monocytes counts differed in patients without complication as compared to those with two adverse in-hospital events.

Then we looked at correlations between WBC counts and CRP levels and PDWc. (Table III).

Important finding was presence of correlation between CRP and monocytes level on the 1st STEMI day. After accounting for CRP levels monocytes counts appeared to have weak negative correlation to HCI. No clear correlations were found between the WBC counts

Table V. Properties of laboratory indices in terms of detection of patients who are likely to experience in-hospital complications of STEMI (n=103)

Indicator, 1st day	AUC	Cut-off value	Sensitivity, %	Specificity, %	PPV, %	NPV, %	p
NLR	0,57	> 7 [1]	53,5	65,6	73,8	43,7	>0,1
WBC-PLT- index > 137 units	0,62	> 137	53,5	71,9	77,5	46,0	<0,05
WBC-PLT index >148 Units	0,62	> 148	48,2	84,3	84,9	47,4	<0,005

and platelet heterogeneity (PDWc and P-LCR) on the 1st STEMI day.

Looking for applicability of acquired information in regards of in-hospital complications prediction we have created rather complex risk assessment criterion. This criterion was derived from data on incremental counts of granulocytes on the 1st STEMI day in patients with greater number of complications and reverse association between lymphocytes and monocytes counts and number of adverse event per patient and also included data on PDWc and P-LCR on the 1st STEMI day. These parameters were used to develop WBC-platelet index and also accounted for baseline CRP levels. (WBC-PLT-CRP index): = ((GRA – MON) / LYM) · 10 + PDWc + P-LCR + CRP

Simplified WBC-PLT index which did not contain CRP data:

WBC-PLT index = ((GRA – MON) / LYM) · 10 + PDWc + P-LCR.

It should be noted, that in developed formula WBC data should be inputted either as counts or proportions of these parameters (but not mixing the input types during one calculation).

Further in the 1st group association between HCI and the 1st STEMI day laboratory data derived indices (WBC-PLT index, WBC-PLT-CRP index, NLR, platelet-to-lymphocyte ratio (PLR)) were studied (Table IV)

WBC-PLT-CRP and WBC-PLT indices showed predictably the highest correlation with HCI ($p < 0.0005$ and $p < 0.001$, respectively). NLR and PLR also were associated with HCI. According to ROC analyses WBC-PLT-CRP index had satisfactory sensitivity (73.7%) and specificity (76.5%), high negative predictive value (83.9%) and low positive predictive value (63.6%), ($p < 0.001$) discerning patients at risk of in hospital adverse STEMI outcomes with cut-off parameter of 160 U.

WBC-PLT index had low sensitivity (66,1%), satisfactory specificity (70,8%), low positive and satisfactory negative predictive value (55,7 and 79,0% respectively) (AUC – 0,69, $p < 0,05$) with cut-off parameter of 137 U.

Combination of laboratory data (WBC-PLT-CRP index > 160 U) and echocardiographic assessment (LV EF < 48 %) on the 1st STEMI day were not sensitive (60 %) but highly specific (90 %) for in-hospital adverse outcomes. Specificity was low (68%) if just one of those indicators were present, but sensitivity for detection of patients with possible complications of STEMI was high and comprised 95%.

Acquired data allowed to construct computer-based algorithm for fast in-hospital complications risk assessment available to use already on the first MI day.

Next stage of study was performed prospectively in the 2nd study group of STEMI patients (n=103) which did not differ significantly from the 1st study group in terms of clinical and anamnestic data. We have studied utility of WBC counts obtained on the 1st STEMI day in terms of detection of patients who were more likely to develop adverse in-hospital outcomes. NLR and WBC-PLT ratios were evaluated. We have also assessed sensitivity, specificity of mentioned parameters and calculated most informative cut-off levels for detection of patients with adverse in-hospital course of STEMI. (Fig.2, table V)

In prospective study WBC-PLT- index appeared to be superior to NLR in terms of detection of patients who are likely to experience in-hospital complications of STEMI.

DISCUSSION

Immune inflammatory reactions triggered by STEMI are highly related to the severity of myocardial damage. On the one hand, pro-inflammatory changes in the immune system can contribute to the death of cardiomyocytes. On the other hand, angiogenesis, regenerative immune responses can contribute to the regeneration of damaged myocardium and the restoration of heart function [18].

We noted a few important aspects regarding the use of cellular immunity in the general clinical practice of treatment of AMI patients. Despite the fact that the greater WBC counts were associated with an increased incidence of adverse cardiovascular events in patients with AMI [19], the most informative indicators for assessing the severity of disease and predicting the long-term consequences of treatment (including interventional) were based on the ratio between the different types of leukocytes and platelets [7, 20]. In particular, increased NLR in patients with ACS appeared to be a statistically significant predictor of hospital and 6-month mortality after coronary interventions [21, 22]. It predicts the development of acute stent thrombosis [23], as well as ventricular arrhythmias and no-reflow syndrome in patients with AMI [24].

It is important to understand that the leukocyte pool consists of different subpopulations, the role of which in the pathogenesis of AMI may be opposite and vary at different stages of the disease. Thus, activated neutrophil granulocytes were detected in coronary thrombi in patients with AMI who underwent primary percutaneous coronary intervention [25]. An increase in the granulocyte number is also associated with a higher risk of cardiovascular diseases, such as MI and ischemic stroke. In addition, the greater number of granulocytes is associated with a higher risk of incidence of subclinical atherosclerosis, which was detected as presence of coronary artery calcification [26].

Microvesicles, as neutrophils derivatives, represent another mechanism by which neutrophils enhance inflammatory processes in damaged heart tissue [27]. In contrast, lymphocytes, especially B2 and T helper cells, as elements of an adaptive immune system, can suppress and limit inflammation. Lower lymphocyte count has been associated with the progression of atherosclerosis and adverse clinical outcomes in patients with heart failure and ACS [28, 29].

Monocytes are involved not only in LV remodeling in the post-infarction period, but also play a significant role in the development of complications, including intracardiac thrombosis [30].

In our work, we created a diagnostic marker for the prediction of the risk of nosocomial complications: WBC-PLT- index, which is more informative than the well-known NLR, due to the fact that our indicator takes into

account more parameters of general blood tests and their relationship (especially when assessing the level of C-RB).

STUDY LIMITATIONS

This is a single-center study were routine practice is early revascularization of patients with STEMI and use of up-to-date medical therapy. Revascularization itself may impact inflammatory pathway and most likely to modify inflammatory response which may differ in patients with spontaneous vessel opening on medical therapy and after mechanical revascularization. In addition, due to the insufficient number of patients in the study sample, we combined the complications of AMI with different mechanisms of their onset, which requires further more specific evaluation for each complication in larger sample of STEMI patients.

CONCLUSIONS

1. Quantitative and qualitative parameters of white blood cells, as well as platelet size heterogeneity, assessed on the first STEMI day are informative in terms of discerning patients who are likely to develop in-hospital STEMI complications. This is especially true for combined indicators, which take into account both the leukocyte counts and platelet distribution.
2. We have developed satisfactory risk assessment tool (WBC-platelet index involving the level of C-reactive protein (WBC-platelet-CRP index) > 160 conventional units), which allows to identify high-risk patients with a sensitivity of 67% and specificity of 82%. When combined with the reduced left ventricular EF on the first day of AMI, the developed WBC-platelet index has a sensitivity of up to 95% and a specificity rises to 90% assessing the risk of complications during the

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ORIGINAL ARTICLE

ANTIBIOTIC ASSOCIATIVE DISORDERS OF THE MICROBIOCENOSIS OF THE COLON IN INFANTS WITH ACUTE RESPIRATORY DISEASES

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ABSTRACT

The aim: Study the effect of antibiotics of different groups on the condition of the colon microflora in infants with acute respiratory pathology

Materials and methods: 140 infants with acute respiratory pathology were examined. Clinical, laboratory and instrumental examination, assessment of the functional state of all organs and systems, chest x ray, clinical signs of the colon microbiocenosis violation, analysis of bacteriological examination and immunological studies of local colon immunity (immunoglobulin concentration (sIgA, IgA, IgG, IgM) in coprofiltrates) were done.

Results: The negative effect of antibiotics of different pharmacological groups on the colon microflora state in infants with acute respiratory diseases has been established. The indigenous microflora of the colon is most inhibited by drugs from the group of 3rd generation cephalosporins, aminoglycosides and their combination. While cephalosporins 1-2nd generations, penicillins and macrolides to a lesser extent affect the state of the microbiocenosis of the colon. The use of two courses of antibacterial therapy to a greater extent disrupts the microbiocenosis of the colon in the examined children, compared with one course of therapy. In commune acquired pneumonia and acute complicated bronchiolitis in infants on the background of antibiotic therapy there is a probable decrease in secretory immunoglobulin in coprofiltrate (sIgA), compared with healthy children ($p < 0.05$).

Conclusions: The analysis of the obtained results showed that antibiotic therapy negatively affects not only the condition of the colon microflora in the examined children, but also suppresses humoral factors of local immunity of the colonic mucosa. Key words: digestive tract microbiocenosis, antibiotic therapy, children.

KEY WORDS: children, antibacterial therapy, gastrointestinal tract microbiocenosis

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INTRODUCTION

The human gastrointestinal tract (GIT) is a relatively open biological system through which the macroorganism actively interacts with the microbial world of the environment. The number of microorganisms in the GIT increases in the distal direction, reaching a maximum in the colon (1011 - 1012 bacterial cells per 1 g of content) [1, 2]. That is why most of the scientific work on the study of human microbial ecology is devoted to the study of the composition and functions of the biocenosis of the colon, which is the most densely populated microflora biotope of the GIT. Representatives of 17 famalies, 45 genuses and more than 400 species of microorganisms were found in the colon biotope [2-5].

There are dominated "characteristic" groups of microorganisms in each biocenosis. In many literary sources they are called differently - obligate, dominant, main, indigenous, resident microflora [3]. It is assigned a leading role in maintaining the symbiotic relationship between the macroorganism and its microbiota. Obligatory microflora performs a regulatory function, counteracts the population of the habitat by random microorganisms and excessive growth of populations of opportunistic pathogens, actively participates in the processes of fermentation, synthesis, detoxification and immunostimulation [3-6].

The causes of the disorder in the microbial status of the body are extremely diverse. It is known that the microecology of the intestine can be influenced by the nature of food, age, season, state of the environment. Unfortunately, the evolution of medicine and pharmacy does not always pay attantion to maintain a friendly symbiosis of human with his microflora. Most modern treatments adversely affect the physiological characteristics of the biocenosis, primarily by inhibiting the beneficial saccharolytic microflora and increasing the population level and aggressive properties of opportunistic pathogens [1, 2, 7, 8]. However, the strongest negative effect on the human microbial ecosystem is manifested by the use of massive, inadequate antibiotic therapy. Unfortunately, there are no antibacterial agents that act exclusively on pathogenic bacteria without affecting the indigenous flora. This requires a rational, reasonable and adequate approach to the use of antibacterial therapy. In addition, there is a need to study the effect of antibiotic therapy on the normal microflora of the colon.

THE AIM

The aim of the research was to study the effect of different antibiotics groups on the state of the colon microflora in infants with acute respiratory diseases.

MATERIALS AND METHODS

There were examined 140 infants with commune acquired pneumonia (CAP) and acute complicated bronchiolitis (ACB). All children were hospitalized in the infants department of Vinnitsa Regional Children's Clinical Hospital. The study included infants who received antibiotic therapy prior to admission to the hospital without using probiotics. Children were divided into two groups: the first group included 80 infants (57.1%) with CAP and the second group consisted of 60 children (42.9%) with ACB. There was a control group consisted of 30 healthy infants.

The work began after obtaining the consent of the patient and his parents to participate in the study in compliance with the provisions of the UN Convention on the Child Rights. The research materials do not contradict the International Code of Medical Ethics (1983) and the laws of Ukraine, meet the basic bioethical norms of the Helsinki Declaration, adopted by the General Assembly of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedicine (1977).

For the diagnosis and treatment of CAP we used British Thoracic Society guidelines for the management of community acquired pneumonia in children (2011) and Clinical practice guidelines of the management of community-acquired pneumonia in infants and children older than 3 months of age of the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America (2011). For the diagnosis and treatment of ACB we used The American Academy of Pediatrics guidelines on the diagnosis, treatment, and prevention of bronchiolitis in children aged 1 to 23 months (2016) and NICE recommendations for *diagnosis and management of bronchiolitis in children (2015).*

All children who were involved in the study underwent thorough clinical, laboratory and instrumental examination, assessment of the functional status of all organs and systems, chest radiography. In addition, an assessment of the clinical picture of the violation of the microbiocenosis of the colon, analysis of bacteriological examination to determine the microbiological landscape and analysis of immunological studies of local immunity of the colon in the study groups. The state of local immunity of the colon in the examined children was determined using the immunological method. The concentration of immunoglobulins (sIgA, IgA, IgG, IgM) in coprofiltrates was determined.

Statistical processing of the study results was performed by conventional methods of variation statistics using IBM SPSS statistic (12th edition).

One of the leading places in the treatment of CAP and ACB is occupied by etiologic therapy with antibiotics, taking into account the age of the child and the conditions of its infection. The analysis of previous antibiotic therapy in the examined patients on admission to the hospital showed that 30 children (37.5%) with CAP received the 3rd generation cephalosporins. Penicillins and penicillin with clavulanic acid were given to 11 children of the first group (13.8%). However, aminoglycosides and the combination of cephalosporins with aminoglycosides were given to the same number of children in this group - respectively 9 patients (11.2%). Also, the same number of children in this group (10.0%) received 1st generation and 2nd generation cephalosporins. Only 5 children of the first group (6.3%) received macrolides.

We also analyzed antibiotic therapy in children of the second group. Thus, almost the same number of children in this group were used 3rd generation cephalosporins - 11 children (18.3%), penicillins - 10 children (16.7%) and macrolides - 10 children (16.7%). At the same time, 9 (15.0%) children in this group received 1st generation cephalosporins and 8 children (13.3%) – 2nd generation cephalosporins. Also 7 children in second group (11.7%) used aminoglycosides and 5 children (8.3%) used the combination of cephalosporins and aminoglycosides.

RESULTS

Since, as mentioned above, the most common cause of colonic dysbiosis in infants with CAP and ACB is antibiotic therapy, the clinical manifestations of impaired colonic microbiocenosis depending on the pharmacological group of drugs were further studied.

Thus, we found that dyspeptic and pain syndromes occurred in all patients treated for the underlying disease with 3rd generation cephalosporins, aminoglycosides, and a combination of cephalosporins and aminoglycosides. There was a significant difference with children used penicillins, macrolides and cephalosporins 1st and 2nd generations ($p < 0,05$) in the incidence of these syndromes.

According to stool character, diarrhea was probably more often diagnosed in children receiving aminoglycosides than in those treated with penicillins, macrolides and 1st generation cephalosporins ($p < 0.05$). At the same time, normal stool was observed with a significantly higher frequency in children who had macrolides, compared with patients treated with penicillins and 3rd generation cephalosporins ($p < 0.05$). In addition, constipation was diagnosed with a significantly higher frequency in children treated with penicillins, cephalosporins 1st and 2nd generations, compared with children who received aminoglycosides ($p < 0.05$).

Dermato-intestinal syndrome was diagnosed with a significantly higher frequency in children treated with macrolides and 1st generation cephalosporins compared with children who had combination antibiotic therapy ($p < 0.05$). Estimation of the frequency of bowel movements during the day in the examined children did not reveal a significant difference between groups with different antibiotic therapy ($p > 0.05$).

Analysis of the nature of stool consistency revealed that liquid stools were diagnosed in patients receiving combination antibacterial therapy for the underlying disease with a significantly higher frequency ($p < 0.05$). At the same time, soft stools were significantly more frequent in children used macrolides and 3rd generation cephalosporins, compared with children treated with 2nd generation cephalosporins ($p < 0.05$).

Subsequently, the results of microbiological examination of feces in the examined children were analyzed, taking into account the obtained antibiotics or their combinations (Fig. 1). The level of bifidobacteria was significantly lower in

Table I. The concentration of immunoglobulins in coprofiltrates in the examined children depending on the pharmacological group of antibacterial therapy

Immunoglobulin	Immunoglobulin concentration in serum, M±m							
	Control group	Penicillin	Macrolide	Cephalosporin 1st generation	Cephalosporin 2nd generation	Cephalosporin 3rd generation	Aminoglycoside	Combination of Cephalosporin and aminoglicoside b
	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l
sIgA	0,79±0,04	0,19±0,11*	0,06±0,01*4	0,18±0,09*	0,27±0,10*	0,28±0,18*	0,06±0,02*4	0,09±0,03*
IgA	0,05±0,01	0,07±0,03	0,09±0,04	0,09±0,04	0,06±0,02	0,09±0,03	0,05±0,03	0,05±0,02
IgG	0,59±0,05	0,27±0,08*	0,16±0,02*	0,15±0,03*	0,15±0,03*	0,17±0,04*	-	0,13±0,01*
IgM	-	0,02±0,01	0,02±0,01	0,03±0,01	0,03±0,01	0,08±0,021234	0,08±0,011234	0,07±0,011234

Notes: * - probable differences in the indicators of the control group (p <0,05). 1 - probable differences in the indicators of children receiving penicillins (p <0,05). 2 - probable differences in the indicators of children received macrolides (p <0,05). 3 - probable differences in the indicators of children received cephalosporins of the 1st generation (p <0,05). 4 - probable differences in the indicators of children received cephalosporins of the 2nd generation (p <0,05).

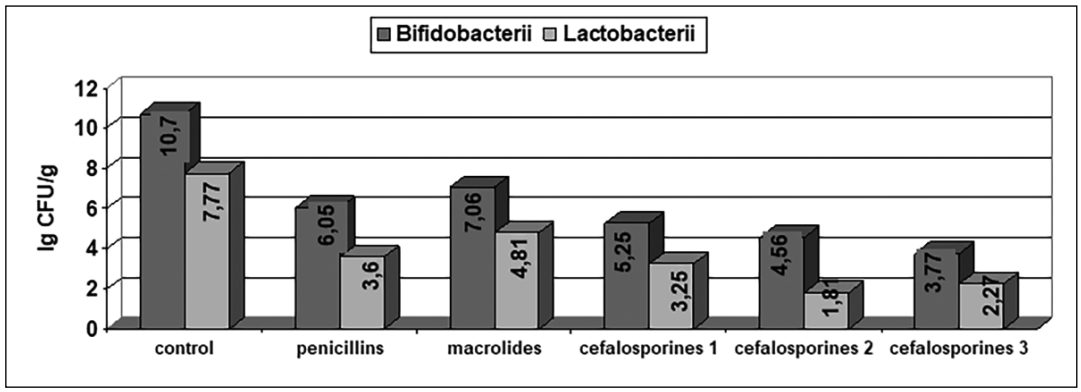

Fig. 1. Results of microbiological examination

children received 3rd generation cephalosporins and a combination of cephalosporins and aminoglycosides, compared with infants used penicillins and macrolides (p <0.05). In addition, the concentration of bifidobacteria was significantly lower in children received 2nd generation cephalosporins, compared with those treated with macrolides (p <0.05).

Analysis of the lactobacilli amount in the colon showed a probable decrease of their level in children received 2nd and 3rd generation cephalosporins, combination antibacterial therapy, compared with those treated with penicillins, macrolides and aminoglycosides (p <0.05). In addition, the concentration of lactobacilli was significantly lower in children received combination antibacterial therapy compared with infants treated with 1st generation cephalosporins (p <0.05).

The study of the *Escherichia coli* level in the examined infants depending on the antibacterial therapy showed a probable decrease only in those who used 2nd and 3rd generation cephalosporins and combination antibacterial therapy, compared with the control group (p <0.05). Analysis of the opportunistic pathogens amount did not reveal a significant difference depending on antibacterial therapy (p> 0.05). Only in children treated with 3rd generation cephalosporins, the amount of Citrobacter was significantly higher than in infants treated with combination antibacterial therapy (p <0.05).

The next part of our research was to analyze the clinical presentation of the colon microbiocenosis disorders in infants with CAP and ACB, depending on the number of antibacterial therapy courses. Analysis of the obtained data indicates that dyspeptic and dermatointestinal syndromes were diagnosed with almost the same frequency in both groups of the study (p> 0.05). However, the frequency of individual clinical manifestations of dyspeptic syndrome probably differed between children with a different number of antibiotic therapy courses. Thus, constipation was observed more often in children received one course of antibiotic therapy, compared with those who received two courses of such treatment (p <0.05). In addition, fecal mucus was observed with a significantly higher frequency in children received two courses of antibiotic therapy (p <0.05).

The pain syndrome was also diagnosed with a higher frequency in infants after two courses of antibiotic therapy (p <0.05), compared with those who had only one course. Analysis of the frequency of bowel movements showed that children who underwent one course of antibiotic therapy, in most cases (p <0.05) were diagnosed with stool frequency 0-1 times a day, compared with those who received two courses (p <0.05). After assessing of the consistency of feces were found that solid stools observed more often in

children after one course of antibiotic therapy ($p < 0.05$). The frequency of other clinical symptoms did not have a significant difference between children with different number of antibiotic therapy courses ($p > 0.05$).

Subsequently, during a microbiological study of feces in the examined children, depending on the number of courses of antibacterial therapy, was found that two courses of antibacterial therapy is more likely suppress the amount of bifidobacterias than in children received one course of antibiotic therapy ($p < 0.05$). At the same time, the amount of lactobacilli and *Escherichia coli* probably did not depend on the number of antibacterial therapy courses ($p > 0.05$). Hemolytic type of *Escherichia coli* was sown in higher concentrations in children received two courses of antibacterial therapy, compared with the infants after one course of such treatment ($p < 0.05$).

One of the parts of our study was to analyze local immunity humoral factors of colon depending on the pharmacological group of antibacterial therapy. Probable decrease of the sIgA concentration in coprofiltrate in both groups compared with the control group ($p < 0.05$) were found. In addition, the level of sIgA in the coprofiltrate was significantly higher in children received 2nd generation cephalosporins, compared with the infants treated with macrolides and aminoglycosides ($p < 0.05$). However, the sIgA level in the examined children coprofiltrate probably did not depend on the pharmacological group of antibacterial therapy they treated with ($p > 0.05$) (Table I).

The concentration of IgG in the coprofiltrate was probably reduced in both groups of the study, compared with the control group ($p < 0,05$). However, IgG was not detected in the coprofiltrate of children treated with aminoglycosides. At the same time, the level of IgM in the coprofiltrate was significantly lower in children treated with penicillins, macrolides, 1st and 2nd generation cephalosporins, compared with infants treated with 3rd generation cephalosporins, aminoglycosides and combination antibacterial therapy ($p < 0.05$).

DISCUSSION

The intestinal microflora relative to the influence of various factors of the external environment, but its compensatory capabilities is not immiscible. There are many etiological factors that can cause a microbiocenosis of the colon. But the leading role in the development of microbiocenosis disorders of the large intestine belongs to antibacterial therapy (antibiotic violations) [9-12]. Most antibiotics used for therapeutic purposes are violated in the process of evolution associative relations between intestinal microorganisms [9, 12]. As numerous studies show, the number of antibiotics accumulated in the contents of the colon, many times exceeds the minimum inhibitory concentrations for a large number of representatives of indigenous microflora [10, 13].

The evaluation of the large intestine microbiocenosis was based not only on the results of the study of feces microflora, but also on the peculiarities of its clinical manifestations. An overview of modern literary sources showed that the most inherent clinical characteristics of antibiotic-sized violations of the large intestine may be dyspeptic, pain and dermatinestinal syndromes [10, 12].

The obtained results of our work show the occurrence of not only diarrhea but also constipation and alternation of diarrhea and constipation on the background of antibiotic therapy. At the same time, constipation was diagnosed with a significantly higher frequency in children on the background of treatment with penicillins and 1st and 2nd generations cephalosporins.

The analysis of modern literature sources demonstrates the development of antibiotic-associated diarrhea (AAD) against the background of antibacterial therapy. The incidence of AAD in the population varies between 5–35% of cases and depends on the patient's age and type of antibiotics [14]. AAD is considered to be predominantly nosocomial pathology (20–25%), which in some cases can reach 60% [1]. However, recently there has been a tendency to increase the frequency of this complication among outpatients [6, 15, 16]. The use of any antibiotics can cause diarrhea, especially those that affect anaerobes. However, more frequent intake of aminopenicillins, cephalosporins of II and III generations is associated with a high risk of developing AAD [3].

The development of dyspeptic phenomenon in violation of the colon microbiocenosis depends on the number of courses of antibacterial therapy. However, the symptoms of pain are more common in children received two courses of antibiotic therapy.

The gastrointestinal tract microflora plays an important role in the functioning of the immune system of the body [7, 17, 18]. The secretory form of IgA dominates over immunoglobulins of other classes in the external secretions of our body [9]. sIgA antibodies inhibit bacterial adhesion to the mucosa, neutralize viruses and participate in immune exclusion [9, 19]. In addition, in almost all secretions of the digestive tract, as well as in coprofiltrates, the same immunoglobulins are found as in serum (IgA, IgG, IgM). However, their concentration in the secretions is slightly lower than in the serum under the influence of local factors. These immunoglobulins enter the secretions of the digestive tract as a result of transudation from serum [2]. Normal sIgA synthesis contributes to the resistance of children to diseases whose pathogenesis is associated with the attachment of pathogens to mucous membranes [9]. Therefore, reducing the concentration of sIgA in coprofiltrates may contribute to the development of various pathologies.

The state of local immunity of the mucous membrane of the colon in examined children was evaluated by humoral factors, namely at the levels of secretory immunoglobulin A and general immunoglobulins (IgA, IgG, IgM) in faces.

The analysis of the obtained results showed that antibiotic therapy has a negative effect not only on the condition of the colon microflora in the examined children, but also suppresses humoral factors of local immunity of the colon mucosa. This fact completely coincides with the clinical observations of modern scientists, where it is indicated that the suppression of the microflora of the colon by antibacterial drugs is a significant increase in the activity of intestinal enzymes. Activation of intestinal enzymes leads to degradation of labile immunoglobulins and loss of antibody activity.

Further in-depth study of the impact of multicomponent probiotics on the state of microbiocenoses of the child's body will help optimize treatment and prevention approaches in the prevention and treatment of antibiotic-associated disorders in pediatrics.

CONCLUSIONS

There are clinical (dyspeptic, pain and dermatointestinal syndromes) and microbiological signs of violation of the microbiocenosis of the colon in infants with CAP and ACB on the background of antibiotic therapy.

The negative effect of antibiotics of different pharmacological groups on the state of the colon microflora in infants with acute respiratory diseases is present. The indigenous colon microflora is mostly inhibited by antibacterial drugs from 3rd generation cephalosporins, aminoglycosides groups and their combination. The using of two courses of antibiotic therapy greater extent disrupts the colon microbiocenosis in the examined children.

There is a probable decrease in secretory immunoglobulin in coprofiltrate (sIgA) in infants with CAP and ACB on the background of antibiotic therapy compared with healthy children ($p < 0.05$).

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ORIGINAL ARTICLE

POSTSTROKE FATIGUE AND MOTOR RECOVERY AFTER ISCHEMIC STROKE

DOI: 10.36740/WLek202205218

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ABSTRACT

The aim: To determine the effect of post-stroke fatigue on the motor recovery after ischemic stroke.

Materials and methods: The study was conducted on the basis of the department of vascular neurology of the Uzhhorod Central City Clinical Hospital during the six months of 2020. We examined 92 patients who suffered a hemispheric ischemic stroke in the early recovery period.

Results: At the end of therapy, improvement of motor function was observed in patients in both groups. We received a significant improvement($p<0,05$) in the patients of the main group on the SSS and 6-point scale of muscle strength. We found that a high score on the FAS showed a significant correlation with the worst dynamics of recovery on the NIHSS, Scandinavian scale of stroke and 6-point scale of muscle strength ($p <0,05$).

Conclusions: According to the results of the study, it was found that the presence of post-stroke fatigue significantly impairs the improvement of motor functions in patients with hemispheric ischemic stroke, patients who had more severe fatigue had worse recovery rates after stroke.

KEY WORDS: ischemic stroke, paresis, post-stroke fatigue

Wiad Lek. 2022;75(5 p2):1328-1330

INTRODUCTION

Poststroke fatigue is a common symptom in patients. A systematic review and meta-analysis found marked variabilities in estimates of poststroke-fatigue prevalence, ranging from 25% to 85%[1]. Poststroke fatigue is generally qualitatively different from fatigue experienced before stroke, as the former can be exacerbated by stress and physical exercise, and generally responds well to rest and adequate sleep[2,3]. Prospective study has reported that, at 6 months after stroke, approximately half (51%) of all patients complained of fatigue[4]. Regarding the duration of fatigue after stroke, acute fatigue can last up to 6 months, whereas the chronic type can persist in 40% of patients after 2 years[2]. Several studies have reported poststroke fatigue to be an independent predictor of shorter survival, institutionalization, poorer functional outcome, and greater dependency for activities of daily living along with instrumental activities of daily living[2] Moreover, in young patients, poststroke fatigue has been reported to be a determinant to resuming work, independent of physical disability or cognitive deficit[5]. In addition, in patients aged 18 to 50 years, poststroke fatigue has been associated with a poor functional outcome, as assessed by the modified Rankin Scale (odds ratio, 4.0; 95% CI, 1.6–9.6)[6].

THE AIM

The aim was to determine the effect of post-stroke fatigue on the motor recovery after ischemic stroke.

MATERIALS AND METHODS

The study was conducted on the basis of the department of vascular neurology of the Uzhhorod Central City Clinical Hospital during the six months of 2020. We examined 92 patients who suffered a hemispheric ischemic stroke in the early recovery period. The criteria for inclusion in the study were: age from 40 to 74 years, verified diagnosis of ischemic stroke of hemisphere localization, absence of severe somatic diseases, poststroke fatigue(the average score on the FAS more than 22 points). General clinical examination, neuroimaging examination (computed tomography, magnetic resonance imaging of the brain), neuropsychological examination using a battery of tests: the Scandinavian scale of stroke(SSS), NIHSS, Scale for assessing muscle strength (Peak L. M. And Braddom R, 1996), the Ashworth scale for assessing the tone of muscles and the Fatigue Assessment Scale (FAS) were used.

Rehabilitation was carried out according to the protocol in the early recovery period during the month with the use of physical therapy, occupational therapy, constraint-induced movement therapy, physiotherapy treatments.

Statistical calculations are performed using the standard statistical package "Microsoft Excel 2010". Differences at $p<0.05$ (95% significance level) and at $p<0.01$ (99% significance level) were considered statistically significant.

RESULTS

We examined 92 patients who suffered a hemispheric ischemic stroke in the early recovery period(the first 3

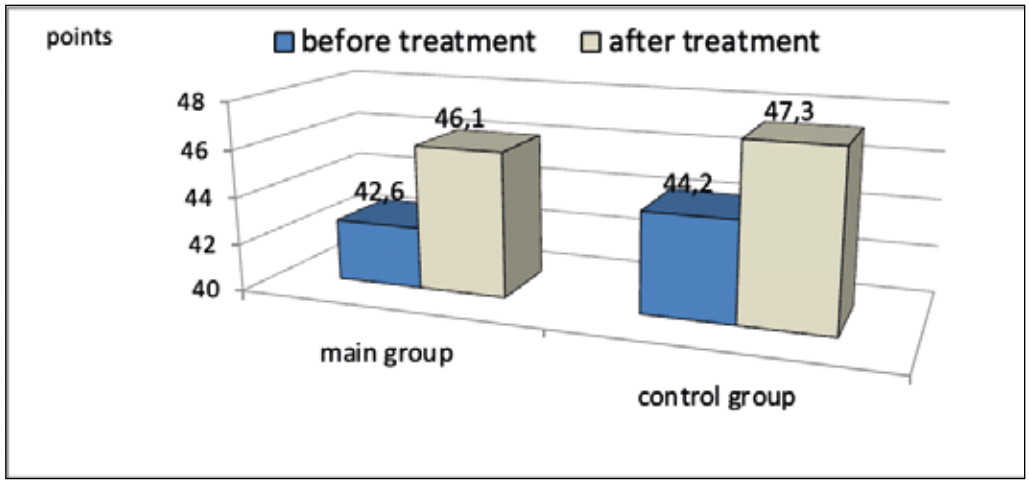

Fig. 1. Dynamics of the functional state of patients in Scandinavian scale stroke

Table I. The effect of poststroke fatigue on motor recovery after ischemic stroke

Scales	FAS	
	the main group	the control group
Scandinavian scale of stroke	r=0,51, p=0,0018	r=0,48, p=0,0016
NIHSS	r=0,38, p=0,0095	r=0,43, p=0,0018
6-point scale of muscle strength, hand	r=-0,43, p=0,0017	r=0,39 p=0,0014
6-point scale of muscle strength, leg	r=0,34, p=0,005	r=0,38, p=0,004
Ashworth's scale, hand	r=0,13, p=0,22	r=0,22, p=0,13
Ashworth's scale, leg	r=-0,10, p=0,49	r=0,21, p=0,14

months). The average age of patients was 59,1 ± 1,1 years. Analyzing the location of brain damage, it was found that 49 patients (54%) had an ischemic stroke of the left hemisphere, and 43 patients (46%) - right hemisphere. The patients were randomly divided into two groups of 46 patients. In the main group physical therapy, occupational therapy, constraint-induced movement therapy, physiotherapy treatments were used, in the control group we used the same methods, except constraint-induced movement therapy.

Before the start of rehabilitation therapy, the mean NIHSS score in patients of the first group was 13.3 ± 2.8 points, in patients of the control group - 12.5 ± 3.7 points. The Scandinavian scale of stroke in patients in the main group had an average score of 42,6 ± 0,8 at baseline and 44,2 ± 0,7 points in the control group(fig. 1).

According to the 6-point scale of muscle strength, for the hand the average score for patients in the first group was 2.9 ± 0.7 points, for patients in the control group 3.0 ± 0.8 points. According to Ashworth's scale, the muscle tone in the parectic limb of the patients in the main group before the study was 2.4 ± 0.8 points, and in the control group patients - 2.2 ± 0.6 points. According to the the Fatigue Assessment Scale average score for patients in the first group was 29,1± 3.7 points, in the control group - 28,2± 2.9 points.

At the end of therapy, improvement of motor function was observed in patients in both groups. We received a significant improvement($p<0,05$) in the patients of the main group on the SSS and 6-point scale of muscle strength.

We found that a high score on the FAS showed a significant correlation with the worst dynamics of recovery on the NIHSS, Scandinavian scale of stroke and 6-point scale of muscle strength ($p <0,05$)(Table I)

DISCUSSION

The current study was aimed to determine the effect of post-stroke fatigue on the motor recovery after ischemic stroke. Poststroke fatigue is a common symptom that can have debilitating effects. Fatigue is defined as a feeling of lack of energy, weariness, and aversion to effort [1]. Fatigue is common after stroke with a prevalence ranging from 16% to 72% [1–2] depending on the population studied and whether patients with mood disorders are included or excluded. Moreover, up to 40% of stroke survivors report it as their worst or one of their worst symptoms[1-2]. The

chronic type can persist in 40% of patients after 2 years [2]. Our study was conducted in patients who suffered a hemispheric ischemic stroke in the early recovery period during the six months of 2020. To detect fatigue in patients we used the FAS – a scale to assess post-stroke fatigue. This scale is the most frequently used scale in patient with stroke[3]. Approximately half (51%) of all patients complained of fatigue at 6 months after stroke[4]. Poststroke fatigue to be an independent predictor of poorer functional outcome and greater dependency for activities of daily living. In our study according to the the Fatigue Assessment Scale average score for patients in the first group was 29,1± 3.7 points, in the control group - 28,2± 2.9 points. To detect disorders of the nervous system we used the Scandinavian scale of stroke(SSS), NIHSS, Scale for assessing muscle strength (Peak L. M. And Braddom R, 1996), the Ashworth scale. Before the start of rehabilitation therapy, the mean NIHSS score in patients of the first group was 13.3 ± 2.8 points, in patients of the control group - 12.5 ± 3.7 points. According to the 6-point scale of muscle strength, for the hand the average score for patients in the first group was 2.9 ± 0.7 points, for patients in the control group 3.0 ± 0.8 points. Rehabilitation was carried out according to the protocol in the early recovery period during the month with the use of physical therapy, occupational therapy, constraint-induced movement therapy, physiotherapy treatments. At the end of therapy, improvement of motor function was observed in patients in both groups. We received a significant improvement($p<0,05$) in the patients of the main group on the SSS and 6-point scale of muscle strength. A number of studies suggest that in patients poststroke fatigue to be a determinant to resuming work, independent of physical disability or cognitive deficit [2-7]. Our study also showed that the patients who had more severe fatigue had worse recovery rates after stroke.

CONCLUSIONS

According to the results of the study, it was found that the presence of post-stroke fatigue significantly impairs the improvement of motor functions in patients with hemispheric ischemic stroke, patients who had more severe fatigue had worse recovery rates after stroke.

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ORIGINAL ARTICLE

METHODS FOR QUANTITATIVE ASSESSMENT OF COLLAGEN ACCUMULATION PROCESSES IN DAMAGED TRAUMATIC INTEGUMENTARY TISSUES

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ABSTRACT

The aim: To study the level of expression of type I, III collagen under conditions of traumatic damage to integumentary tissues and to develop quantitative criteria for collagen formation processes for choosing the volume of reconstruction of a wound defect.

Materials and methods: Studies were performed for 62 victims. Clinical group 1 (n = 16) – patients with wound surface sizes of the body surface area, group 2 (n = 32) consisted of patients who received integumentary tissue defects due to or together with damage osteoarticular apparatus of the limb, group 3 (n = 14) – patients with combined trauma.

Results: In the skin samples of patients of groups 1 and 2 of the affected groups, a statistically significant difference was established between the indicators of the relative accumulation area of type I, type III collagen (W = 675, p = 0.01 and W = 697, p = 0.03, respectively). In patients of groups 2 and 3, when analyzing relationships, a direct moderate strength was observed between the areas of collagen deposition of type I, type III (r_s = 0.52 and 0.54). Collagen deposition coefficient (Kd) was used as a quantitative criterion for assessing the prognosis of the results of restoration of integumentary tissues according to the proposed scale, which made it possible to obtain postoperative complications in only 3.2 % of cases.

Conclusions: A positive treatment result taking into account the quantitative criterion (Kd) was obtained in 96.8 % of the examined patients.

KEY WORDS: trauma, collagen expression, wound surface, reparative capabilities, damaged integumentary tissues, immunohistochemical studies

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INTRODUCTION

In the early stages of physiological healing of harmful skin during the formation of granulation tissue, the mechanisms of matrix remodeling are included [1-3]. In addition, as a rule, at the first stage immature and unstable type III collagen is detected, most of which is later replaced by mechanically stable mature and a denser collagen type I [4,5].

However, in traumatic skin injuries, combined injuries, the healing of skin defects can result in pathological scarring [6], leading not only to cosmetic defects, but also to serious functional disorders – anatomical (desmogenic and arthrogenic) deformities, compressions or loss of joint mobility.

It is known that only in the early stages after surgery it is possible to carry out effective preventive and pathogenetically justified corrective measures for pathological scarring [7-9]. To develop criteria for evaluating rehabilitation treatment, the features of collagen formation processes in different variants of wound surface healing may be of some interest.

Currently, there are few studies that describe the types of collagen in the collagen fibers of normotrophic and pathological scars and a significant difference between the levels of expression of collagen type I, III in patients with defects of integumentary tissues of traumatic origin [10].

So far, no reliable laboratory tests and diagnostic criteria have been developed to predict the option of healing in the area of injury in the early stages of wound epithelialization, the formation of desmogenic contractures and scar tissue [11,12], and the data obtained can be used in clinical practice as differential and diagnostic criterion in predicting the nature of the wound process and objectively justifying a rational method of reconstructive intervention [13,14].

Therefore, in recent years, much attention has been paid to the study of the level of collagen expression of different types in conditions of physiological regeneration and the formation of pathological scars at skin damage [15,16]. At present, there are few research studying the reparative capabilities of the skin in trauma for the choice of surgical tactics [17,18].

The leading trend in the works of modern specialists is the search and implementation of new methods aimed at intensifying the reparative capabilities of the body and the ability of the skin to reproduce qualitative and quantitative parameters of fibrous structural elements of the dermis, increasing the proliferative activity of fibroblasts [19,20].

THE AIM

The aim was to study the level of collagen type I, III expression in conditions of traumatic damage to integumentary tissues and to develop quantitative criteria

for the processes of collagen formation to select the extent of reconstruction of the wound defect.

MATERIALS AND METHODS

Morphological studies were performed on 62 persons with traumatic tissue damage of the extremities. All injuried were divided into three clinical groups depending on the depth of the injury, the size of the wound and the extent of tissue injury.

Clinical group 1 (n = 16) – patients with wound size greater than 1 % of body surface area and damage to skin and soft tissues below the deep fascia, group 2 (n = 32) consisted of patients who received defects of integumentary tissues due to or together with damage to the musculoskeletal system of the limb, group 3 (n = 14) – patients with combined trauma, accompanied by damage to the vascular-nerve bundles, bones, muscles, partial or complete separation of the limb.

Material for pathomorphological research – pieces of skin 10x10 mm, which were removed during primary surgery, staged necrectomies from around the wound area.

For pathomorphological and immunohistochemical study, skin fragments were fixed in buffered 10 % formalin and embedded in paraffin. Serial sections of 4 μm thickness were made on a precision rotary microtome NM 3600 (MICROM Laborgerate GmbH, Germany), which were placed on SUPERFROSTPLUS adhesive slides by DAKO, Denmark. The main microscopic changes in sections of skin tissue were analyzed in micronutrients stained with hematoxylin and eosin and by Van Gizon.

Immunohistochemical studies using monoclonal antibodies were performed in paraffin sections of the skin according to standardized protocols. After dewaxing and rehydration of the sections, temperature unmasking of the antigens was performed (by heating in a water bath in Tris-EDTA buffer with pH = 9.0) and inhibited the activity of endogenous peroxidase with 3 % hydrogen peroxide solution. After incubation with primary antibodies, the corresponding antigens were imaged with the EnVision FLEX detection system with diaminobenzidine.

Immunohistochemical determination of collagen type I and III accumulation was performed using monoclonal antibodies Rb a-Hu Collagen I, Clone RAH C11 and Rb a-Hu Collagen III, Clone RAH C33 (Imtek, Russia).

To assess the relative area of accumulation of collagen type I and III in skin tissue fragments, the preparations were photographed in 5 fields of view with Canon EOS 1000D digital camera (Japan) in Axioplan 2 microscope (CarlZeiss, Germany) at x 200 and analyzed using Image J. software. For morphometric measurement of the relative area occupied by immunopositive structures, a standard "sensitivity threshold" (Threshold tool) was set in the filtered DAB image channel for automatic segmentation of a digital image into two main types of points – pixels (black and white) followed by the ratio of the number of black dots-pixels (immunopositive zones) to the total number of pixels in the image, expressed as a percentage, according to the following formula:

$$S_{rel} = p_{pos} / p_{tot} * 100\ \%$$

where S_{rel} – the relative area of collagen deposition, expressed as a percentage, p_{pos} is the number of black pixel dots (immunopositive zones), p_{tot} – the total number of pixels in the image.

Statistical processing of quantitative research results was performed on a personal computer using the analysis package in ExcelMicrosoftOffice 2013 and STATISTICA® for Windows 6.0 (StatSoft Inc., license No. AXXR712D833214FAN5). The median (Me), lower and upper quartiles (Q1, Q3) were calculated for the data. The presence of relationships was determined by the Spearman's correlation coefficient, the differences between the groups by the criterion of Kruskal–Wallis, the comparison of the related groups was performed by the Wilcoxon test (W).

RESULTS

In the victims of clinical group 1 in the histopathological examination of tissue sections around the wound area of defects stained with hematoxylin and eosin, we determined the areas with signs of scar tissue formation with areas of thickening of the epidermal layer with no wavy structure of dermal-epidermal combinations and not enough frequent formation of ridges and dermal papillae.

In parallel histochemical examination of these biopsies stained by Van Gizon, in the superficial dermis and in its central areas there were revealed parallel oriented strands and thin ligaments of collagen, that had a reticular structure, in the deeper parts of the dermis found randomly located too thick circles, between which there were clusters of cells, primarily fibroblasts.

Immunohistochemical examination of biopsies using monoclonal antibodies to collagen type I determined its pronounced expression (22.89 (19.84; 26.47) %) and significant accumulation in all layers of the dermis in the form of wavy single pale and intensely colored thin plate-like fiber fragments and thick chaotically intertwined collagen fibers, which were collected in bundles of fibrils.

In a study with monoclonal antibodies to type III collagen, pronounced expression (26.85 (21.91; 30.92)%) was also observed due to a significant accumulation of embryonic collagen in the form of pale and intensely colored disorganized fibrillar strands of different thickness, running in parallel in all layers of the dermis.

When performing photographic digital morphometry of skin biopsies in the examined patients in clinical group 1, it was determined that the minimum value of the relative area of type I collagen deposition is 12.23 (19.84; 26.48) %, the maximum value is 44.34 (19.84; 26); 48) %, the relative area of collagen type I deposition was 22.90 (19.84; 26.48) %.

The minimum value of the relative area of type III collagen deposition in the same samples is 14.66 (21.91; 30.93) %, the maximum is 46.56 (21.91; 30.93) %, the indicator of the relative area of type III collagen deposition was 26.85 (21.91; 30.93)%.

Comparative analysis by Wilcoxon test allowed determining a statistically significant difference between

the area of collagen deposition type I and III in the studied skin fragments of patients in group 1: W = 675, p = 0.01.

That is, it was found that in the structures of the dermis of the examined patients in clinical group 1 with excessive wound surface and soft tissue injury below the deep fascia there is a denser accumulation of type III collagen than type I collagen (Fig. 1), which according to other authors is found only in immature connective tissue in the early stages of repair and indicates injury to the skin over a large area [4,5].

Pathohistological examination of skin biopsies of patients in clinical group 2 also showed a violation of the histostructure of the skin and was characterized by flattening of the dermal-epidermal combination and the absence of skin appendages (hair follicles, sebaceous and sweat glands).

Immunohistochemical examination of these samples revealed replacement of the papillary and reticular layers of the dermis with scar tissue with excessive accumulation of collagen, which according to many experts is a feature of pathological scarring [2]. Among the densely spaced collagen bundles there were found single clusters of fibroblasts with focal infiltration of lymphocytes.

In the analysis of digital images of the studied skin fragments with immunopositive zones in patients of clinical group 2, the intensive accumulation of collagen type I, III was determined (23.64 (20.30; 28.15) %) and 22.73 (18.25; 25.86) %, respectively) in the form of immunopositive closely spaced light and dark brown individual thin fibers, which were organized in nodes and located parallel to the skin surface preferably. According to other studies, this pattern is characteristic of the onset of hypertrophic or keloid scar tissue.

The relative area of type I collagen deposition in the skin sections of patients in group 2 ranged from 14.76 (20.30; 28.15) % and 35.89 (20.30; 28.15)%, the median was – 23.64 (20.30; 28.15) %.

The relative area of type III collagen deposition ranged from 8.66 (18.25; 25.86) % to 39.55 (18.25; 25.86) %, and the median was 22.73 (18.25; 25.86) %. From the results of various studies it is known that dense collagen type I is always detected at the final stage of scar tissue formation, with its excessive accumulation indicates sclerotic changes in the dermis [1]. It is important to understand the treatment tactics that the synthesis of this protein begins in the first week of wound surface repair and is a consequence of the degradation of embryonic collagen type III [4].

Comparative analysis of collagen formation processes confirmed the presence of a statistically significant difference between the area of collagen type I and III deposition in skin biopsies of patients in clinical group 2: W = 697, p = 0.03.

Thus, it was found that a significant accumulation of mature collagen type I (Fig. 2) in the studied skin fragments of the victims of group 2 indicated untimely formation of mature scar tissue or was the result of initial processes of imbalance between synthesis and degradation of extracellular matrix proteins and led to pathological tissue formation in the form of hypertrophic or keloid scar.

At the same time, persistent desmogenic or arthro-desmogenic contractures of the limb or constantly functionally stressed area (soles of the feet, etc.) were developed in a patient.

In the patients of clinical group 3, histopathological examination of skin samples revealed thickening and thinning of the epidermis with a slight formation of dermal papillae, the presence of a significant number of vertically oriented blood vessels and the absence of hair follicles and sebaceous glands.

Immunohistochemical examination of skin samples of the examined patients revealed areas with intensive accumulation of collagen and areas of partial disorganization of collagen fibers.

A weak focal infiltration of single lymphocytes and the formation of immunocytic infiltrates were revealed in some areas of the studied fragments, mainly around the vascular structures of the dermis.

Intensive accumulation of brown-colored collagen fibers type I and III in all dermal layers in 28.22 (18.88; 32.11)% and 28.35 (18.04; 35.56) %, respectively) in skin biopsies of patients of this group during a complex immunohistochemical and digital morphometric study. It was determined that the relative area of type I collagen deposition ranged from 6.04 (18.88; 32.11) % to 38.83 (18.88; 32.11) %, while the relative area of collagen III deposition type ranged from 14.62 (18.04; 35.56) % to 38.72 (18.04; 35.56) %. Differences in the relative area of collagen type I and III deposition in patients of clinical group 3 are statistically insignificant (W = 523; p = 0.08).

Comparative analysis by the Kruskel-Wallis test revealed the absence of a statistically significant difference between the area of collagen type I deposition in skin biopsies of patients in groups 1, 2, 3 (p = 0.19). However, there was a statistically significant difference between the indicators of the area of collagen type III deposition in the studied sections of patients of all clinical groups: the largest indicators of the area of collagen type III deposition were registered in clinical group 3 (p = 0.01; Fig. 3).

The analysis of relationships using Spearman's rank correlation criterion between the respective areas of collagen type I and III deposition showed that patients of clinical groups 2 and 3 showed a direct moderately strong connection (r_s = 0.52 and 0.54). This confirms the fact of normal wound surface repair, when there is a gradual interdependent formation of the main types of skin collagen with gradual degradation of embryonic collagen III and its modification into mature collagen type I at the end of scar formation.

Using correlation analysis in determining the Spearman's ratio in patients with a large wound surface and soft tissue damage below the deep fascia (clinical group 2), found a statistically insignificant correlation of weak strength (r_s = 0.22) between the accumulation of collagen type I and III, which is noted in Table I.

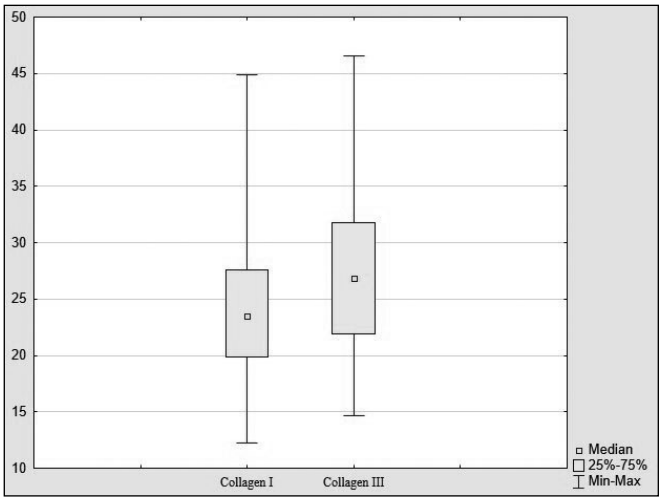

Fig.1. Distribution of the area of collagen type I, III deposition in skin fragments of the examined patients in clinical group 1. Units of measurement – %.

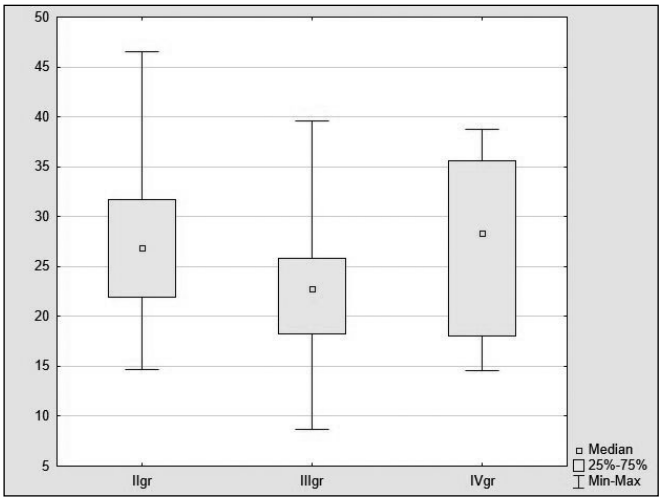

Fig. 2. Comparison of the area of collagen type I, III deposition in skin sections of the studied patients in clinical group 2. Units of measurement – %.

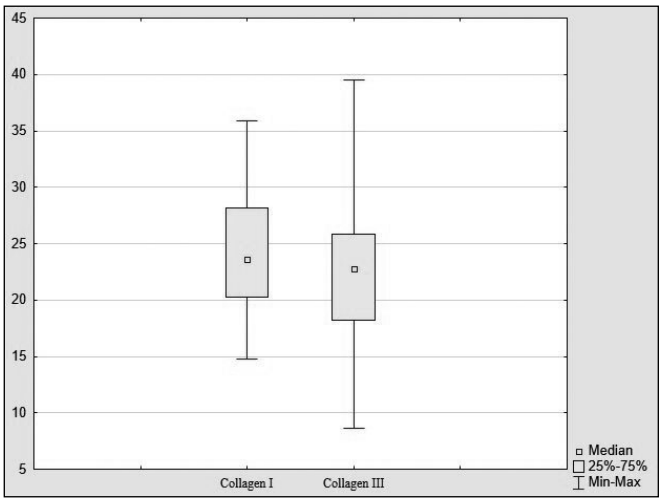

Fig. 3. Comparison of the area of type III collagen deposition in skin biopsies of patients in clinical groups1, 2, 3. Units of measurement – %.

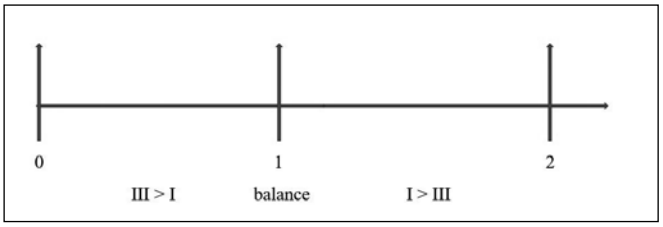

Fig. 4. Diagnostic scale for assessing the accumulation of collagen type I, III in patients with injuries of the skin and soft tissues.

Based on the indicators obtained of estimating the area of collagen type I, III deposition in skin fragments of the examined patients, collagen deposition coefficients were calculated for all clinical groups, ie for different types of integumentary tissues damage (Table II), which were used as quantitative criteria for assessing the prognosis of the results of skin and soft tissue repair, according to the formula:

K_d = Me I/ Me III,

where K_d – collagen deposition rate in the dermis,
Me I – median area of collagen type I accumulation,
Me III – median area of collagen type III accumulation.

The analysis of collagen accumulation in different types of damage to integumentary tissues allowed to create a scale for assessing the processes of collagen formation in order to predict the outcome of the restoration of the injured skin cover (Fig. 4).

According to the scale, the further K_d from 1 (balance), the greater the imbalance of collagen accumulation processes in damaged integumentary tissues.

Postoperative complications occurred in 62 (3.2 %) cases among 62 patients who used this study technique – in 1 case in female patient of clinical group 1 with K_d = 0.85 without additional reconstruction and in 1 patient of group 3 with K_d = 0.89. The patient required additional restorative intervention.

Thus, histopathological examination of skin biopsies from the around wound area in all clinical groups of patients revealed thinning of the epidermal layer with the absence of a normal wavy dermal-epidermal combination, excessive accumulation of collagen in all dermal layers in the form of parallel-oriented individual and collected in bundles fibrils in the superficial sections and unsystematically located thicker collagen bundles in the deep layers of the dermis, between which there were clusters of fibroblasts;

- in the skin samples of patients of clinical groups 1 and 2 found a statistically significant difference between the relative area of accumulation of collagen type I, III (W = 675; p = 0.01 and W = 697; p = 0.03, respectively);
- in patients of groups 2 and 3 in the analysis of relationships using the criterion of Spearman's rank correlation, a direct moderate-strength relationship was observed between the areas of collagen type I, III deposition (r_s = 0.52 and 0.54);
- collagen deposition coefficient (K_d) for different types of damage was used as a quantitative criterion

for assessing the prognosis of the results of recovery of integumentary tissues on the proposed scale, which confirmed the postoperative complications only in 3.2% of cases.

DISCUSSION

Conducted analysis of professional literature and our own clinical material convinced us that the earliest possible use of reconstructive surgical technologies from the moment of traumatic injuries is an effective method of treating patients with isolated and multiple injuries of various anatomical structures to restore the shape and function of the body. The use of microsurgical techniques and complex flaps in the acute period of trauma is not always possible and necessary, especially when it comes to severe combined polystructural changes with damage to the great vessels, nerves, bones, super-large defects of the skin and underlying soft tissues. During such a period, the main task of the surgeon is to close the wound surface quickly in order to avoid the spread of wound infection and to qualitatively restore the vital structures of the body [2,5].

The developed complex diagnostic program for studying the reparative capabilities of the skin makes it possible to predict and select the volume of restorative intervention in the early stages after injury [6]. With sufficient individual capabilities of the body to repair integumentary tissues, which we defined as universal quantitative indicators, and the presence of a defect not in functionally active areas (joints), it is not always necessary to expand the volume of the operation, and the main task is early rehabilitation of the patient and reducing the consequences of traumatization of body structures.

In traumatic skin injuries, the healing of skin defects can result in pathological scarring [6], leading not only to cosmetic defects, but also to serious functional disorders – anatomical deformities, compressions or loss of joint mobility.

It is known that only in the early stages after surgery it is possible to carry out effective preventive and pathogenetically justified corrective measures for pathological scarring [7,9]. To develop criteria for evaluating rehabilitation treatment, the features of collagen formation processes in different variants of wound surface healing may be of some interest.

Based on the collagen deposition area indices, the deposition coefficients (K_d) had been calculated for different types of damage, which was used as a quantitative criterion for predicting the results of the repair of the covering tissues (type II - K_d = 0,85, type III - K_d = 1,04, type IV - K_d = 0,89) and a prognostic scale was developed.

Accordingly, the further K_d is from 1 (balance), the greater the imbalance of collagen accumulation processes in the damaged covering tissues. In 2 (3,2 %) cases, postoperative complications occurred in patients who had used this technique. In 1 case the patient of group 2 with K_d = 0,85 and 1 patient of group 4 with K_d = 0,89.

CONCLUSIONS

1. Analysis of the results of a comprehensive morphological study of skin fragments affected with defects of integumentary tissues of traumatic origin revealed that the worst prognosis for the reconstruction of damaged integument and restoration of function were patients with damage over 1 % of body surface and simultaneous injury of bones, muscles, arteries and nerves
2. A positive result of treatment according to a certain quantitative criterion (K_d) was obtained in 96.8 % of the examined patients.

Prospects for further research are in the field of evaluating the effectiveness of surgical interventions to eliminate various types of defects of integumentary tissues and the choice of method of reconstruction of the wound defect

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ORIGINAL ARTICLE

CHARACTERISTICS OF DEVELOPMENT OF EMOTIONAL BURNOUT SYNDROME IN FAMILY DOCTORS AND THEIR CORRECTION METHODS

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ABSTRACT

The aim: Among the adverse psychological changes associated with medical work, a significant place belongs to the emotional burnout syndrome, which significantly affects a doctor's social functioning. The purpose of the study was to determine the behavioural activity types and to study the muscular sensitivity threshold of family doctors with the formed emotional burnout syndrome.

Materials and methods: We examined 83 female family doctors with diagnosed emotional burnout syndrome. The treatment was carried out with the help of the Taiji-Quan eastern wellness system.

Results: The analysis of indicators of the behavioural activity revealed before treatment in 72,5% of women in the intervention group and in 69,8% of women in the control group ($p>0,05$) signs of the high probability of type A behaviour. After a three-month course of treatment, the percentage of manifestations of type A behaviour in women in the intervention group decreased from 72,5% to 40,0% ($p 0,05$). When examining the features of muscular tone at the beginning of treatment, it was found that 69,9% of women in the intervention group and 65,1% of the control group had a high degree of muscle blocks ($p>0,05$). After treatment, the percentage of women with a high degree of muscle blocks decreased to 37,5% ($p 0,05$) in the control group.

Conclusions: The taiji-Quan treatment made it possible to significantly reduce type A behaviour and to decrease the amount of "muscle blocks".

KEY WORDS: family doctors, emotional burnout syndrome, type A behavioral activity, muscle blocks, Taiji-Quan

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INTRODUCTION

Among the adverse psychological changes associated with medical activity, a significant place belongs to an emotional burnout syndrome (EBS), which significantly affects the overall mental health and social functioning of doctors [1,2].

An important prerequisite for the development of EBS is the individual-psychological characteristics of the physician, and one of the important factors that leads to emotional burnout is when individual characteristics do not match the requirements of "person-person" type professions [3]. In particular, EBS often occurs in individuals with impulsive behavior and high behavioral activity in various fields, with a tendency to compete and dominate (so-called "type A" behavior), which is one of the causes of accumulation of negative emotions and physical exhaustion [4]. Emotional burnout syndrome is associated with constant emotional overload and constant interpersonal communication [5].

One of the most effective psychotherapeutic methods in the treatment of psychosomatic and mental pathology is body therapy techniques using a variety of techniques aimed at eliminating local muscle tension, muscle relaxation and restoration of normal muscle kinematics [6].

Among the methods of active body therapy, Taiji occupies a special place - a traditional Chinese health-improving system that combines physical exercises and meditative techniques based on thousands of years of practice [7]. A number of studies have confirmed that Taiji has a positive effect on general physical and psychological health, sleep quality [8], can be used to improve the condition of patients with cardiovascular pathology [9], metabolic syndrome [10], diseases of the musculoskeletal system [11] and some oncological diseases [12].

The positive impact of Taiji on the psycho-emotional sphere, confirmed in a number of studies, is extremely important; while the therapeutic effect of Taiji was proved to a wide range of psychopathological symptoms, including affective disorders, cognitive impairment and severe endogenous mental illness, as well as improving the quality of life of patients with mental disorders [13-19].

However, the therapeutic potential of Taiji as an effective tool for the normalization of the psychoemotional condition, treatment and rehabilitation of patients with affective, neurotic and psychosomatic disorders remains insufficiently studied [20]. Such studies are of particular relevance against the background of the growing scientific and practical interest in the search for non-drug alternatives to

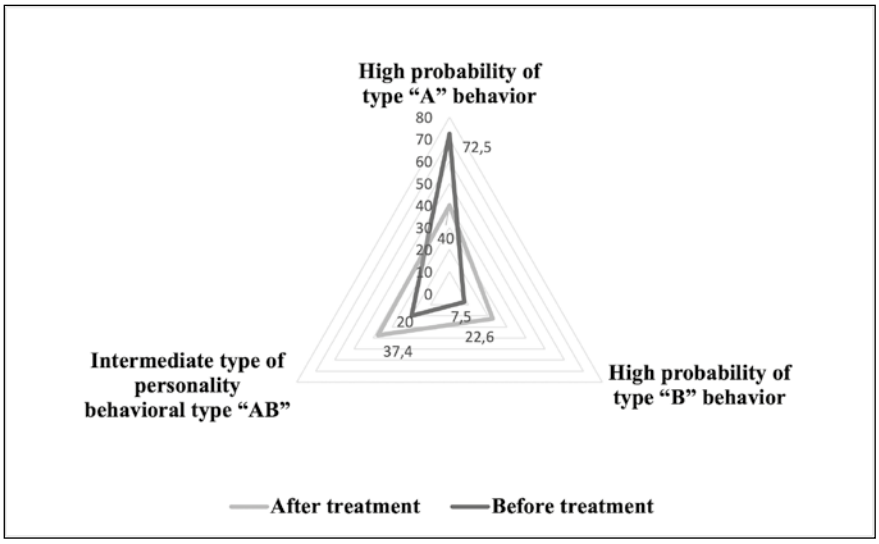

Fig. 1. Characteristics of indicators of the level and the type of behavioral activity in family doctors with EBS before and after the treatment

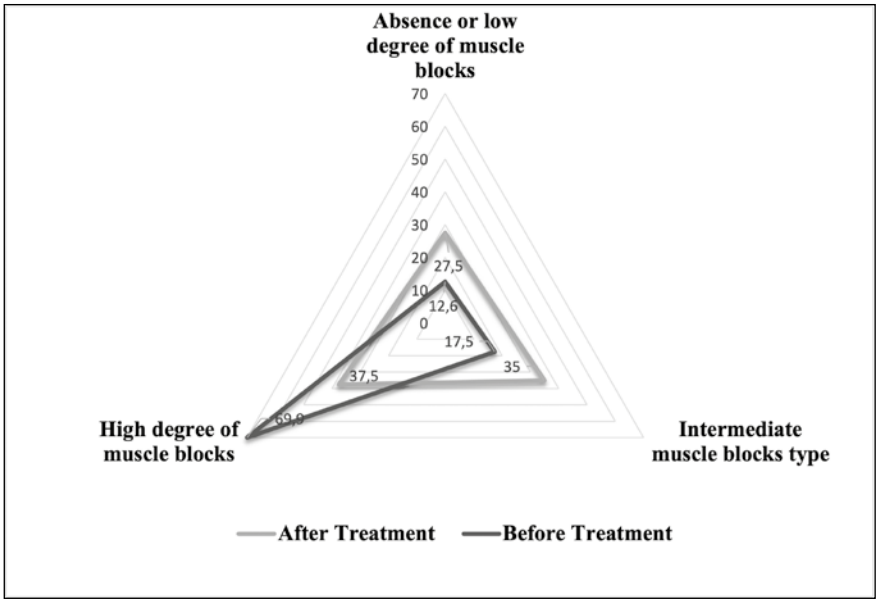

Fig. 2. Characteristics of muscle tone in family doctors with EBS before and after the treatment

therapeutic interventions for borderline mental pathology and mental disorders of the non-psychotic register.

THE AIM

The purpose of the study was to determine the behavioural activity types and to study the muscular sensitivity threshold of family doctors with the formed emotional burnout syndrome.

MATERIALS AND METHODS

In accordance with the principles of biomedical ethics, we selected and examined 83 female family doctors who worked in health care facilities in Vinnytsia and Vinnytsia region with diagnosed EBS. The average age of examined persons was 44,6 ± 12,9 years. Quantitative analysis of the degree of emotional burnout was performed using the V. V. Boyko's method of diagnostics of the emotional burnout level [21]. At the same time, the score of less than 61 points was regarded as a non-formed EBS, and the score of 61 points and above was considered as a formed EBS. A Jenkins' modified test was used to determine the level and the type of behavioral activity [22]. Muscle system tension level was measured using Fehr T. modified technique [23].

Statistical and mathematical analysis of the divergences was carried out using Fisher's exact test. A statistical divergence of 95,0% ($p<0,05$) was considered acceptable.

All examined persons were divided into two groups. The first (intervention group - IG) consisted of 40 women with Taiji-Quan basic training (24 standard exercises) for 3 months at a rate of 3 sessions, 1,5 hours per week. The training program also included discussion of somatic and psychic sensations that occurred during the training session in the treatment group, explaining to women IG the causes and mechanisms of EBS, approaches to its elimination, as well as forming a positive attitude to the lessons. The control group (CG) consisted of 43 women who did not practice any wellness system. IG and CG groups were similar in age and social characteristics.

Table I. Comparison of indicators of the level and the type of behavioral activity, determined by the Jenkins' modified test, in family doctors with formed EBS in main and control group before and after the treatment

The level and the type of behavioral activity	Before treatment		After treatment		p
	abs.	%	abs.	%	
Intervention group					
High probability of type "A" behavior	29	72,5	16	40,0	<0,01
High probability of type "B" behavior	3	7,5	9	22,6	<0,05
Intermediate type of personality behavioral type "AB"	8	20,0	15	37,4	<0,05
Total	40	100,0	40	100,0	-
Control group					
High probability of type "A" behavior	30	69,8	29	69,9	>0,05
High probability of type "B" behavior	5	11,6	5	11,6	>0,05
Intermediate type of personality behavioral type "AB"	8	18,6	9	18,5	>0,05
Total	43	100,0	43	100,0	-

Table II. Comparative characteristics of muscle tone features in family doctors with formed EBS in main and control group before and after the treatment

Level and type of behavioral activity	Before treatment		After treatment		p
	abs.	%	abs.	%	
Intervention group					
Absence or low degree of muscle blocks	5	12,6	11	27,5	<0,01
Intermediate muscle blocks type	7	17,5	14	35,0	<0,05
High degree of muscle blocks	28	69,9	15	37,5	<0,01
Total	40	100,0	40	100,0	-
Control group					
Absence or low degree of muscle blocks	6	14,0	4	9,3	>0,05
Intermediate muscle blocks type	9	20,9	10	23,3	>0,05
High degree of muscle blocks	28	65,1	29	67,4	>0,05
Total	43	100,0	43	100,0	-

RESULTS

The analysis of indicators of the level and the type of behavioral activity, determined by Jenkins' modified test, revealed before treatment in 72,5% of women in the intervention group and in 69,8% of women in the control group (p>0,05) signs of high probability of type A behavior, which characterized by an aggressive-compulsive pattern of response. Such people spend a lot of time at work, actively working and gaining authority, fighting for goals with methods of hostility to others [21]. On the other hand, the proportion of women with high probability of psychologically favorable type B behavior in IG and CG was 7,5% and 11,6% respectively (p>0,05). An intermediate "AB" type of behavioral type of personality was found in 20,0% of women in IG and in 18,6% of women in CG (p>0,05) (Table I, Figure 1).

After a three-month course of treatment, the percentage of manifestations of type A behavior in women in the IG decreased from 72,5% to 40,0% (p<0,01) and in the CG – from 69,8% only to 69,9% (p>0,05). At the same time, the proportion of women with high probability of type B behavior in IG increased from 7,5% to 22,6% (p<0,05), and in CG remained unchanged (11,6%, p>0,05). The proportion of women with a high probability of type AB behavior in IG increased from 20,0% to 37,4% (p<0,01), and in CG – slightly decreased from 18,6% to 18,5% (p>0,05) (Table I, Figure 1).

During the study of the characteristics of muscle tone it was found that before the start of treatment the absence or low degree of muscle blocks was found in 12,6% of women in IG and 14,0% of women in CG (p>0,05), intermediate type of muscle blocks – 17,5% and 20,9% respectively (p>0,05), high level of muscle blocks – 69,9% and 65,1% respectively (p>0,05) (Table II, Figure 2).

After the treatment, the percentage of women in IG with no or low degree of muscle blocks increased to 27,5% (p<0,01) and decreased to 9,3% (p>0,05) in CG. In IG the proportion of women with intermediate type of muscular blocking increased to 35,0% (p<0,05), and in CG - slightly increased to 23,3% (p>0,05). At the same time, the proportion of women with high degree of muscle blocks decreased in IG to 37,5% (p<0,01) and increased in CG to 67,4% (p>0,05).

DISCUSSION

The data obtained in our study allow us to speak about the complex positive influence of Taiji on the psychoemotional condition of medical workers with EBS. A significant de-

crease in the percentage of individuals with a high risk of type A behavior, associated with increased levels of aggressiveness, emotional lability, irritability, dominance indicates a general normalization of the psychoemotional condition, a decrease in the manifestations of negative emotions and an improvement in the level of mental functioning. This correlates with data from studies by Zhang J. et al. (2018); Liu J. et al. (2018), who revealed a reduction in depression and anxiety associated with Yang-style Taiji exercises [15,16]. Our data also confirm the results of the study by Yu et al. (2018), reporting on the positive effects of Taiji on psychological well-being, in particular in reducing stress, anxiety, depression and affective disorders, as well as improving patient self-esteem [13]. The decrease in the level of anxiety and aggressiveness is consistent with the data obtained by S. Gogulla et al. (2012), noting the effectiveness of Taiji in increasing patient confidence and reducing the severity of psychosomatic symptoms [14]. A parallel increase in the proportion of a physician with an intermediate type of type AB behavior, which is characterized by an emotional stability and rationality, and type B behavior, which is characterized by a tendency to think carefully about decisions, low impulsivity and consistency of behavioral reactions, achieved with Taiji, indicates an improvement in the social interaction of patients and is consistent with the results of Liao S. et el. (2019), who report on the potential of Taiji exercise to improve social functioning [17], as well as research results from Ho R.T.H. et al. (2016) [18] and Sungkarat S. et al. (2017) [19], who revealed a positive effect of Taiji on cognitive functioning and improvement of the psychological condition of patients.

At the same time, it should be noted that there are currently no systematic studies of the effectiveness of Taiji exercises for patients with EBS. EBS is a separate clinical and social phenomenon that has common features with other neurotic, affective and psychosomatic disorders, but at the same time is distinguished by the specificity of pathogenetic and pathopsychological mechanisms, the uniqueness of clinical manifestations and it require special therapeutic, rehabilitation and preventive approaches. In this context, our research is of great scientific and practical importance, which allows us to fill the research vacuum in this area, to provide information on the use of the modern Taiji health-improving system in eliminating the harmful psychological consequences of EBS.

CONCLUSIONS

1. Emotional burnout syndrome developes more often in persons with a predominance of characterological features, inherent in persons with type A behavior and, in addition to psychopathological symptoms, also appears in increased muscle tone.
2. The proposed method of EBS correction with the use of the therapeutic technique of Taiji-Quan combined with psychotherapeutic discussion can significantly reduce muscle tone and change the percentage of manifestations of type "A" behavior, which is an indicator of normalization of psychological and somatic health.
3. The system of correction of psychological and somatic health on the basis of the therapeutic technique of Taiji-Quan may be recommended for use in the range of medical and rehabilitation activities for EBS.

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ORIGINAL ARTICLE

CLINICAL AND IMMUNOLOGICAL FEATURES OF ROTAVIRUS INFECTION IN CHILDREN INFECTED WITH EPSTEIN-BARR VIRUS

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ABSTRACT

The aim: To identify clinical and immunological features of acute rotavirus gastroenteritis occurring against the background of Epstein-Barr virus infection.

Materials and methods: The study involved examination of 56 children. Of them, 33 children (1 group) did not have a background infection and 23 patients (2 group) suffered from rotavirus infection on the background of the latent form of Epstein-Barr virus infection. Children in these groups were compared by gender, age, severity of the disease and other parameters. Quantitative data were presented as mean and standard deviation (M±SD). Differences at $p < 0.05$ were considered statistically significant.

Results: The data suggest that the presence of background Epstein-Barr virus in children with rotavirus infection leads to later hospitalization, lower temperature response rates, lower frequency of vomiting at the onset of the disease, and longer duration of fever and diarrhea. At the same time, in children infected with Epstein-Barr virus, the relative content of CD8+ T lymphocytes dominated both in the acute period of the disease and in the period of convalescence against the background of reduced relative content of CD16+, CD22+ T lymphocytes and IgM in the period of early convalescence.

Conclusions: The study allowed to reveal the influence of latent EBV infection on the clinical and immunological parameters of rotavirus gastroenteritis.

KEY WORDS: intracellular pathogens, latent Epstein-Barr infection, rotavirus gastroenteritis

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INTRODUCTION

In the structure of morbidity of the gastrointestinal tract of infectious nature, rotavirus infection (RVI) occupies one of the leading places among infants and young children [1, 2]. In the available literature there is a large number of studies devoted to the assessment of the clinical picture of RVI [3]. However, recently there have been publications in which scientists point to changes in clinical symptoms and the course of RVI, which researchers associate with a number of causes of exogenous and endogenous nature [2, 3]. A number of authors consider mass infection of children with Herpes viruses to be one such reason, which can affect the patient's immune response [4, 5]. Herpes viruses, which children become infected with in the first years of life, also include Epstein-Barr virus (EBV) [6, 7]. The mechanisms of interaction between EBV and the immune system of the macroorganism have been studied by many scientists, but their conclusions differ [4, 5, 8]. Some authors point to the lack of influence of EBV on the immune response, others believe that the presence of EBV in a child can lead to suppression of the functional activity of its immunocompetent organs and the development of immunosuppressive state [9-11]. At the same time, some scientists report beneficial, immunomodulatory effects of lifelong interaction between the virus and the immune system, considering the relative uniqueness of EBV-specific $CD4^+$ and $CD8^+$ T-cells to be the evidence of the latter, which is the ability to activate in response to other pathogens in physiologically relevant method and determine the development of heterologous cross-immunity [12].

Changes in the clinical picture of RVI in recent years, differences in the results of studies of the immune response of children in this disease and the possible impact of EBV, became an argument in favor of conducting research in this direction.

THE AIM

The purpose of the study was to identify clinical and immunological features of rotavirus infection in children infected with Epstein-Barr virus.

MATERIALS AND METHODS

The study involved supervision of 56 children aged one to three years, patients with moderate and severe intestinal infections of rotavirus etiology, for which they received appropriate treatment in Kharkiv Regional Children's Infectious Diseases Clinical Hospital. Of them, 33 children (first group) did not have a background infection and 23 patients (second group) suffered from RVI on the background of the latent form of EBV. The criteria of the inclusion of children in the study groups were the diagnosis of acute intestinal infection caused by rotavirus for the first group, and both

Table I. Clinical symptoms of RVI and their duration in children uninfected and infected with EBV (M±SD)

Clinical symptoms	Group 1 (n=33)	Group 2 (n=23)	p
Maximal temperature, °C	39.04±0.48	38.5±0.31	<0.001
Duration of febrile temperature, days	3.00±0.86	4.26±0.54	<0.001
Duration of subfebrile temperature, days	1.00±0.70	2.00±0.52	<0.001
Maximal daily frequency of vomiting	4.21±2.23	2.38±1.43	0.002
Duration of vomiting, days	1.36±0.74	1.17±0.83	0.330
Maximal daily frequency of diarrhea	5.39±1.54	5.21±1.38	0.817
Duration of diarrhea, days	3.57±0.79	4.52±0.84	<0.001
Duration of catarrhal signs, days	3.36±1.03	3.60±0.65	0.223

Table II. Indices of cellular and humoral links of the immune response in the examined patients in the period of early convalescence (M ± SD)

Indices, reference norms	Group 1 (n=33)	Group 2 (n=23)	P
Lymphocytes, abs. (N – 4-9 *109/L)	3.52±0.63	3.58±0.56	0.652
CD 3+, % (N – 50 - 80%)	63.66±6.15	63.96±5.84	0.782
CD 3+, abs (N – 1,0 - 1,5 *109/L)	1.97±0.86	2.30±0.47	0.194
CD 4+, % (N – 33 - 46%)	39.38±3.09	37.83±2.67	0.081
CD 4+, abs (N – 0,4 – 0,8*109/L)	1.21±0.51	1.35±0.22	0.653
CD 8+, % (N – 17 – 30%)	29.48±3.8	34.52±3.01	<0.001
CD 8+, abs (N – 0,2 - 0,4 *109/L)	0.91±0.38	1.24±0.24	<0.001
CD 4+ / CD 8+ (N – 1,4 - 2,0)	1.34±0.12	1.10±0.14	<0.001
CD 16+, % (N – 12 - 23%)	19.9±3.88	16.65±2.44	<0.001
CD 16+, abs (N – 0,1 - 0,25*109/L)	0.62±0.30	0.60±0.14	0.211
CD 22+, % (N – 17 - 31%)	33.38±4.35	28.04±2.75	<0.001
CD 22+, abs (N – 0,1-0,3*109/L)	1.03±0.44	1.00±0.19	0.104
Ig A, g/l (N – 0,2 - 1,0 g/l)	0.90±0.20	0.83±0.20	0.254
Ig M, g/l (N – 0,19 - 1,46 g/l)	1.86±0.43	1.15±0.23	<0.001
Ig G, g/l (N – 4,53 – 9,16 g/l)	10.34±0.84	10.71±0.94	0.063

Table III. Indices of cellular and humoral links of the immune response in the examined patients in the acute period of the disease (M±SD)

Indices, reference norms	Group 1 (n=33)	Group 2 (n=23)	P
Lymphocytes, abs. (N – 4-9 *109/L)	5.11±1.67	5.21±1.45	0.696
CD 3+, % (N – 50 - 80%)	60.61±6.63	60.00±7.42	0.688
CD 3+, abs (N – 1,0 - 1,5 *109/L)	3.09±1.04	3.14±0.98	0.874
CD 4+, % (N – 33 - 46%)	28.94±5.02	31.04±5.17	0.130
CD 4+, abs (N – 0,4 – 0,8*109/L)	1.48±0.56	1.60±0.45	0.310
CD 8+, % (N – 17 – 30%)	23.67±3.29	28.57±3.82	<0.001
CD 8+, abs (N – 0,2 - 0,4 *109/L)	1.23±0.50	1.50±0.48	0.058
CD 4+ / CD 8+ (N – 1,4 - 2,0)	1.23±0.20	1.10±0.24	0.001
CD 16+, % (N – 12 - 23%)	26.15±5.39	22.17±3.65	0.002
CD 16+, abs (N – 0,1 - 0,25*109/L)	1.32±0.43	1.15±0.34	0.140
CD 22+, % (N – 17 - 31%)	21.09±2.89	20.26±2.05	0.356
CD 22+, abs (N – 0,1-0,3*109/L)	1.09±0.43	1.07±0.36	0.796
Ig A, g/l (N – 0,2 - 1,0 g/l)	0.42±0.11	0.43±0.10	0.500
Ig M, g/l (N – 0,19 - 1,46 g/l)	0.80±0.27	0.73±0.15	0.260
Ig G, g/l (N – 4,53 – 9,16 g/l)	7.82±0.75	8.17±0.74	0.086

acute intestinal infection caused by rotavirus and latent EBV infection for the second group. Verification of RVI diagnosis was carried out by isolating rotavirus antigen from the feces of patients by enzyme-linked immunosorbent assay (ELISA) and the corresponding IgM antibodies in the blood. The presence of latent EBV infection was established in the presence of specific antibodies IgG to EBV in the serum of children and in the absence of IgM (ELISA) and nucleic acid (PCR) of herpesviruses types 1, 2, 4, 5, 6. The criteria of the exclusion: positive results for bacterial and viral pathogens of intestinal infections, except rotavirus; latent herpesviruses infection (positive specific IgG to herpesviruses types 1, 2, 5, 6) or active herpesviruses infection (positive result to specific IgM or/and nucleic acid of herpesviruses types 1, 2, 4, 5, 6) and children with somatic background diseases.

Children in these groups were compared by gender, severity of the disease and age. The first group included 15 (45,5%) girls and 18 (54,5%) boys, the second group included 14 (60,9,%) girls and 9 (39,1%) boys, $\chi 2 = 1.290$, $p = 0.256$. At the same time, 15 (45,50%) children from the first group and 12 (52,2%) from the second group suffered from moderate form of RVI, and 18 (54,50%) patients from the first group and 11 (47, 8%) from the second, suffered RVI in a severe form $\chi 2 = 0.245$, $p = 0.620$. The average age in group 1 was 23,3±5,2 months, and in group 2 was 26,1±7,2 months, $p = 0.060$.

The Vesicari score was used to determine the severity of rotavirus gastroenteritis. This scale takes into account the frequency and duration of diarrhea and vomiting, the level of temperature response, as well as the degree of dehydration of the patient and the level of medical care. Each of these indicators is evaluated in points, and the sum of points is then used to assess the severity of the disease. Mild disease is diagnosed with a score of <7, moderate from 7 to 10 points, and severe with a score of > 11.[13].

The generally accepted laboratory tests (general blood test, general urine analysis, coprogram and bacteriological examination of feces), given in the Order of the Ministry of Health of Ukraine No. 803 of 10.12.07 "Protocol for the treatment of acute intestinal infections in children" were carried out. In addition to generally accepted laboratory tests, patients underwent immunological tests. The content of lymphocytes CD3 +, CD4 +, CD8 +, CD16 +, CD22 + in blood serum was also determined by immunofluorescence method using monoclonal antibodies (GRANUM, Ukraine) during the time course of the disease (1-2 and 8-10 days). The immunoregulatory index (IRI) was calculated as the ratio of the relative CD4 + / CD8 + content. The content of immunoglobulins of class A, M, G (IgA, IgM, IgG) (g/l) of blood serum was determined using two-site enzyme-linked immunosorbent assay (sandwich method) (GRANUM, Ukraine).

Statistical analysis was performed using IBM SPSS 25.0 statistical software package. Continuous data were reported as M±SD. Significance between two independent variables was calculated using Mann-Whitney U-test, significance between two related variables was calculated using Wilcoxon matched-pair signed-rank test. Critical α-error value was 0.05, thus the result was considered statistically significant in $p < 0.05$. Exact two-sided p-values were reported.

RESULTS

The clinical picture of RVI, in almost 100% of cases, was characterized by fever, recurrent vomiting, frequent liquid stools without pathological impurities. Moreover, 78% of patients had catarrhal manifestations (serous discharge from the nose, coughing, hyperemia of the mucous membrane of the posterior pharyngeal wall). Assessment of literature data and the results of our own observations suggest preservation of the overall clinical symptoms of RVI in children at present. However, the severity of the above clinical manifestations of the disease and their duration was probably determined by the background condition of the child, including, in our opinion, the presence of infection of the patient with EBV (Table I).

A total of 56 children were examined. Group 1 included 33 children, of whom 15 (45.5%) had moderate to severe and 18 (54.5%) had severe condition ($p = 0.602$). Group 2 included 23 patients, of whom 12 (52.2%) had moderate and 11 (47.8%) severe disease ($p = 0.835$). The mean age of patients in Group 1 was 23.30 ± 5.25 months and 26.08 ± 7.20 months in Group 2 ($p = 0.060$). There were no gender differences among the comparison groups, $p = 0.602$ in Group 1 and $p = 0,297$ in Group 2.

Assessment of temperature response showed that in patients of Group 2 the average maximum body temperature at the onset of the disease was significantly lower than in patients of Group 1 (38.5 ± 0.31 C, 39.04 ± 0.48 C, $p < 0.001$). However, the duration of febrile fever in them was almost one and a half times (4.26 ± 0.54 days and 3.00 ± 0.86 days, $p < 0.001$), and subfebrile temperature (2.00 ± 0.52 days and 1.00 ± 0.70 days, $p < 0.001$) almost 2 times longer than in Group 1 children.

Evaluation of the parameters of multiplicity and duration of vomiting revealed that patients infected with EBV (Group 2) had almost 1.5 times lower daily multiplicity of vomiting, compared with uninfected (2.38 ± 1.43 and 4.21 ± 2.23 $p = 0.002$). The duration of vomiting was almost the same (1.36 ± 0.74 days and 1.17 ± 0.83 days, $p = 0.330$).

As can be seen from Table I, the maximum frequency diarrhea in children of both groups in the acute period was almost the same. However, in patients infected with EBV (Group 2) the duration of diarrhea was significantly longer than in patients with mono-RVI (Group 1) (4.52 ± 0.84 days and 3.57 ± 0.79 days, $p < 0.001$).

When studying the parameters of catarrhal syndrome, no differences were found in the frequency of their occurrence among the compared groups. This syndrome was identified in 26 patients (78.79%) in Group 1 and in 18 patients (78.26%) in Group 2, ($p = 0.335$). Also there were no differences in duration of catarrhal syndrome (rhinitis, pharyngitis), were found (3.36 ± 1.03 days and 3.60 ± 0.65 days in Group 1 and in Group 2, respectively).

The revealed clinical differences in the course of RVI on the background of EBV infection, such as lower temperature response rates and lower vomiting rate, at the onset of the disease, probably became a factor in later hospitalization

of these children from the onset of the disease compared with patients in Group 1 (2.3 ± 0.8 days and 1.8 ± 0.8 days, p = 0.05).

Given the presence of clinical differences in RVI in the comparison groups, the study also involved an assessment of the relative and absolute content of subpopulations of T- and B-lymphocytes and immunoglobulins of classes A, M, G in the blood of children of both groups during the disease.

As can be seen from Table II, the total number of lymphocytes (abs) and their subpopulations of $CD3^+$ (%, abs), $CD4^+$ (%, abs) in the acute period of the disease among the comparison groups had no significant differences. In contrast, Group 2 patients were found to have a significantly higher relative content of $CD8^+$ T-lymphocytes than in Group 1 patients (28.57 ± 3.82 and 23.67 ± 3.29, p < 0.001), and the absolute content of $CD8^+$ cells tended to increase relative to children of the first group.

The immunoregulatory index (IRI) in patients infected with EBV was significantly lower than in the first group (1.10 ± 0.24 and 1.23 ± 0.20, p = 0.001). Besides, Group 2 patients, in the acute period of the disease, were shown to have a significant decrease in the relative content of $CD16^+$ T-lymphocytes (22.17 ± 3.65 and 26.15 ± 5.39 p = 0.002). Indices of the humoral part of the immune response did not have significant differences among children of the comparison groups at the onset of the disease.

During the period of early convalescence there were no significant differences in the absolute content of lymphocytes and $CD3^+$ (%, abs), $CD4^+$ (%, abs) and their subpopulations.

When analyzing the levels of $CD8^+$ T-lymphocytes in the blood of patients in the study groups, it was found that in patients infected with EBV, in the period of early convalescence there was a significant increase in both their relative and absolute content (p <0.001).

Due to the increased level of $CD8^+$ T-cells, patients with latent EBV infection developed an imbalance causing a significant decrease in the IRI during early convalescence, compared with patients of Group 1 (1.11 ± 0.14 and 1.34 ± 0.12%, p < 0.001).

The relative content of $CD16^+$ T-lymphocytes during the disease remained significantly lower in patients with background EBV infection than in children without it (16.65 ± 2.44 and 19.9 ± 3.88, p < 0.001).

The relative level of $CD22^+$ T-cells in the period of early convalescence increased in both groups, but in children on the background of EBV infection (Group 2) there was a significantly lower relative level (28.04 ± 2.75 and 33.38 ± 4.35, p < 0.001). The absolute content of $CD22^+$ T-cells had no significant differences among the comparison groups. Besides, Ig M content in Group 2 children in the period of early convalescence relative to Group 1 patients was significantly lower (1.15 ± 0.23 and 1.86 ± 0.43, p < 0.001).

DISCUSSION

There is significant number of scientific researches devoted to the study of effect of EBV on the immune response and course of other infectious diseases. However, we did not find the researches which study effects of EBV on RVI.

The current study demonstrates the effect of EBV on the clinical and immunological parameters of RVI. Thus, in the acute period of the disease, the presence of Epstein-Barr virus infection in children with RVI leads to their later hospitalization, lower temperature response rates, and lower frequency of vomiting in the onset of the disease. To our knowledge, such effects can reduce the risk of dehydration and contribute to effective oral rehydration in the prehospital phase, and possibly even reduce the number of hospitalizations. In the period of early convalescence in children with latent EBV infection, a longer persistence of fever and diarrhea was observed. This finding coincides with the data of other scientists who studied the effect of EBV on the course of intestinal and respiratory diseases, although these authors describe significant manifestation of clinical symptoms predominantly in acute period of the disease [13, 14].

Analysis of the interrelations of immune parameters in children infected with EBV showed significantly higher relative and absolute count of $CD8^+$ T-lymphocytes respectively during whole course of disease and in the period of convalescence. The increase in the level of $CD8^+$ T-lymphocytes is probably due to the stimulation of $CD8^+$ cell synthesis by herpesviruses, which is described in researches [6, 15, 16]. Some authors also point to the immunomodulatory properties of $CD8^+$ T-lymphocytes, due to their ability to be activated in response to other pathogens [12]. It is possible that the activity of T-cytotoxic lymphocytes was the cause of a less pronounced clinical manifestation at the beginning of the disease. Decreased IRI, in our opinion, is due to the increased content of $CD8^+$ lymphocytes, which are directly used in the calculation of IRI. Although according to the literature, the approximation of IRI to 1.0, found in children of second group, indicated a tendency to immunodeficiency [15].

Both in the acute period and in the period of early convalescence, a reduced level of $CD16^+$ T-lymphocytes was found in children with latent EBV infection, which is probably associated with the inhibitory effect of herpesviruses on NK-cells, which was described in other studies [17, 18]. The lack of significant differences in the absolute count of $CD16^+$ T-lymphocytes necessitates further study of this issue.

In addition, the period of early convalescence in patients infected with EBV is characterized by a delay in activation of $CD22^+$ T-cells on the background of lower than in the control group content of Ig M, which may indicate a delay in activation of the humoral immune response. We hypothesize that these changes could increase the duration of fever and diarrhea in patients of second group.

The data suggest that the presence of EBV infection directly affects the mechanism of the immune response by altering the count of T-lymphocytes, especially cytotoxic, and immunoglobulins, which ultimately contributes to changes in the clinical manifestations of the disease.

CONCLUSIONS

The presence of EBV infection in patients with RVI is associated with less pronounced clinical symptoms of RVI in children in the acute period of the disease.

During the period of early convalescence - increased duration of the main symptoms of rotavirus gastroenteritis

Concerning of the cellular part of the immune system in children infected with EBV, we have not established clear criteria that would indicate the immunosuppressive effect of latent WEB infection on the immune response of the child during acute rotavirus gastroenteritis.

The period of convalescence in children infected with EBV is characterized by a lower content of CD22 + T-cells and IgM than in children without background infection. In our opinion, such differences indicate a delay in the activation of the humoral link of the immune response, and, probably, in combination with other factors, is a factor in the prolongation of clinical manifestations of RVI.

PROSPECTS FOR FURTHER RESEARCH

In our opinion, the clinical and immunological parameters of RVI can be used to create an algorithm for early diagnosis of EBV infection in children with rotavirus gastroenteritis, which will improve certain therapies for these patients and tactics for monitoring convalescents.

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ORIGINAL ARTICLE

EMPIRICAL RESEARCH ON THE FEATURES OF PSYCHOEMOTIONAL STATES OF ADOLESCENT ATHLETES DURING QUARANTINE RESTRICTIONS

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ABSTRACT

The aim: To study the features of psychoemotional states of adolescent athletes during quarantine restrictions, depending on gender, type of sport (individual or team) and the typological profile of the hubristic motivation.

Materials and methods: The empirical sampling was composed of 116 adolescent athletes. During the study, the methods of "Determination of the dominant state" (L. Kulikov), "Indicator of coping strategies" (D. Amirkhan), "Questionnaire on hubristic motivation" (K. Fomenko) have been used.

Results: Gender differences in psychoemotional state and in the choice of coping strategies have been established: the boys have a higher level of calmness and tend to choose a strategy aimed at problem solving, whereas the girls are more unease and tend to avoid problems. It has been found that the psychoemotional state of adolescent athletes of individual sports is more stable compared to adolescent athletes of team sports. The dominant striving for perfection in the profile of hubristic motivation of adolescent athletes has been revealed, which contributes to staying resilient and keeping positive attitude to life situation, as well as the choice of positive strategies for stress overcoming.

Conclusions: Psychoemotional states of adolescent athletes during quarantine restrictions have a number of features. The most vulnerable to quarantine effects are female athletes and adolescent athletes of team sports. The striving for perfection in the typological profile of hubristic motivation has a positive effect on the psychoemotional state of adolescent athletes.

KEY WORDS: psychoemotional state, adolescent athletes, coping strategies, hubristic motivation

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INTRODUCTION

Quarantine restrictions applied during COVID-19 pandemic has affected various spheres of human life. Restrictions in life events from self-isolation to adaptive quarantine have been lasting more than a year and cause an impact on sports activities. Athletes, accustomed to daily training and considerable exercise, found themselves in a situation of uncertainty due to changes in training schedules, withdrawal or postpone of sports competitions at the international, regional and national levels. Adolescent athletes belong to the category of people who are accustomed to spending time actively, constantly gaining new experiences, fighting for a prize-winning place, a competitive environment, etc. Therefore, quarantine restrictions could affect their psychoemotional state, cause anxiety and certain behavioral reactions.

Under the COVID-19 pandemic, the immune system can cause changes at the behavioral and personality levels, leading to heightened suspicion, alertness, conformism, propensity for authoritarian submission [1]. The most well-known psychological signs of quarantine, as the forms of isolation, are sensory deprivation, monotony, uniformity of the interior design, permissive nature, hypodynamia, boredom, which can be the precursors of certain mental discomfort and even depression [2]. Self-isolation and social distancing applied as the anti-epidemic measures act as a stress factor [3], and keeping to coercive quarantine restrictions provokes anxiety, depression, shortness of temper and anger, leading to emotional exhaustion, destructive relationships and alcohol abuse [4]. Age and gender differences have been identified [5] in the emotional response; in particular, older athletes and professional athletes feel more unease and desperate in the above circumstances [6].

The issue of psychoemotional states of adolescent athletes during quarantine restrictions is poorly elucidated in recent studies. Adolescence is characterized by a number of psychological features that can have a significant impact on the psychoemotional state of an individual, especially under the influence of stressors. One of the little studied factors of influence is a hubristic motivation. In the "transgressive concept" of Yu. Kozeletskyi it is interpreted as a steady striving of a human being for reinforcement and heightening of increasing self-esteem and self-importance, as well as a persistent striving for superiority, perfection and expansion of his/her power [7].

We consider hubristic motivation as the striving for self-assertion, self-aggrandizement and reinforcement of self-esteem, which is realized in two ways: through the achievement of perfection and / or superiority [8]. Hubristic motivation is manifested in the behavior of the self-asserting subject in his/her emotional reactions to life situation that contribute to or hinder the achievement of self-respect.

THE AIM

The aim – to study the features of psychoemotional states of adolescent athletes during quarantine restrictions, depending on gender, type of sport (individual or team) and the typological profile of the hubristic motivation.

MATERIALS AND METHODS

The empirical sampling was composed of adolescent athletes (n=116; 51 (44%) girls and 65 (56%) boys) aged 13-14 years, engaged in various sports (swimming, volleyball, basketball, football, freestyle and Greco-Roman wrestling). The adolescents attended Children and Youth Sports Schools with training regimen 6 to 10 trainings a week and have been going in for sports from 3 to 6 years. 48 (41%) and 68 (59%) adolescents have been involved in individual and team sports, respectively.

To study the specifics of the psychoemotional state of adolescent athletes, the method of "Determination of the dominant state" (L. Kulikov, 2003) was chosen [9]. Determination of the dominant type of behavior in stressful situations was carried out according to the method of "Indicator of coping strategies" (D. Amirkhan, 1990) [10]. To determine the typological profile of hubristic motivation, the "Questionnaire of Hubristic Motivation" (K. Fomenko, 2010) was used [8]. The analysis of differences in the distribution of features in independent groups of subjects was performed using non-parametric (N. Kruskal — Wallis) and parametric (Student's t-test) criteria. The procedure of grouping the subjects in their pairwise comparison simultaneously on several indicators was performed using the cluster analysis by the method of k-means. Statistical data processing was performed using the StatSoftStatistica 10 software.

RESULTS

The findings of the study of the gender differences in the dominant states of adolescent athletes have confirmed the existence of significant differences between the male and female adolescents. Thus, the significant differences on two scales of the dominant state have been established: calmness-anxiety ($t = 3,69$; $p<0,0005$) and stability-instability of emotional tone ($t = 2,98$; $p<0,005$). Indicators on the first parameter in boys fluctuated within $48,45 \pm 11,43$ and in girls within $40,76 \pm 1,85$; on the second parameter - $47,74 \pm 9,69$ and $41,78 \pm 11,89$, respectively. The data shows that male athletes have higher level of calmness compared to their female peers.

The female athletes had a higher level of anxiety. Longer emotional distress, the tendency to feel unease in life situations, see the threat to prestige, well-being, no matter how real the reasons are, turned out to be more typical for them compared to male athletes. The male athletes are characterized by higher emotional stability and the ability to more effectively self-regulate behavior and activities compared to the female athletes. Given that girls have higher scores on the anxiety scale, higher emotional stability of boys is quite predictable. Consequently, during quarantine restrictions, female athletes were more vulnerable to anxiety, emotional arousal, and mood swings compared to their male peers.

The findings of the study on the choice of coping strategy by adolescent athletes have established the gender differences (Table I).

It has been found that there are significant differences in the indicators of problem solving ($t = 3,06$; $p<0,005$) and problem avoidance ($t = -4,47$; $p<0,0005$). It has been established that male athletes used such a productive coping strategy as problem solving to reduce the impact of stressors under quarantine restrictions. Female athletes are more disposed to unproductive strategy of avoiding problems in contrast to their male peers. Notably, the comparison between the indicators of coping strategies of adolescent athletes of different gender and the indicators of dominant states has shown that the state of anxiety and emotional instability in girls led them to choose a strategy of problem avoidance. And the state of calmness and emotional stability allow male athletes to make a choice in favor of a problem-solving strategy.

The analysis of the psychoemotional states of adolescents engaged in individual and team sports has shown the significant differences only in the scores of the "stability-instability of emotional tone" scale: indicators in team sports ranged from $43,45 \pm 10,82$, and in individual sports they were in the range of $47,58 \pm 12,23$ ($t = -2,1$; $p<0,05$). Adolescent athletes of individual sports were emotionally more positive; in a state of emotional arousal adequacy was maintained compared to their peers of team sports. An important feature of team sports is the joint success, i.e., the success of the team and the personal success of each teammate. Given the frequent changes in the training regimen, the indefinite duration of quarantine restrictions, changes in team composition, the success of teamwork could be threatened. Athletes of individual sports are more autonomous in sports activities; they could practice certain skills even during self-isolation. The adolescents who are members of the team have experienced more difficulties, since team training was canceled for some time. And in the future there is a possibility of re-imposing restrictions on group training.

To study the role of the motivating factor in the development of certain dominant states of adolescent athletes, typological profiles of hubristic motivation of the subjects have been determined. For this purpose, the cluster analysis was performed by the method of k-means indicators of the striving for perfection and superiority and three cluster profiles have been identified, presented in Fig. 1.

Table I. Indicators of coping strategies of adolescent athletes depending on gender ("girls / boys" groups)

Indicators	Groups of subjects		t	p
	Boys (n =65)	Girls (n = 51)		
Problem solving	26,61±3,74	24,24±4,58	3,06	<0,005
Problem avoidance	20,84±2,99	23,45±3,24	- 4,47	<0,0005

Table II. Indicators of dominant states of adolescent athletes with different typological profiles of hubristic motivation

Indicators of dominant states	Typological profiles of hubristic motivation			H	p
	Dominant striving for perfection (n =37)	Dominant striving for superiority (n =45)	Lower level of hubristic motivation (n = 34)		
Active-passive attitude to life situations	52,68±9,69	49,71±10,96	45,29±10,84	7,48	<0,05
Vigor-despair	50,27±9,27	48,16±9,13	43,85±10,66	7,07	<0,05

Table III. Indicators of coping strategies of adolescent athletes with different typological profiles of hubristic motivation

Indicators	Typological profiles of hubristic motivation			H	p
	Dominant striving for perfection (n =37)	Dominant striving for superiority (n =45)	Lower level of hubristic motivation (n = 34)		
Problem solving	27,53±5,53	25,60±3,64	23,33±4,86	15,42	<0,0005
Search for social support	24,50±5,37	23,36±4,29	20,45±4,78	13,28	<0,005

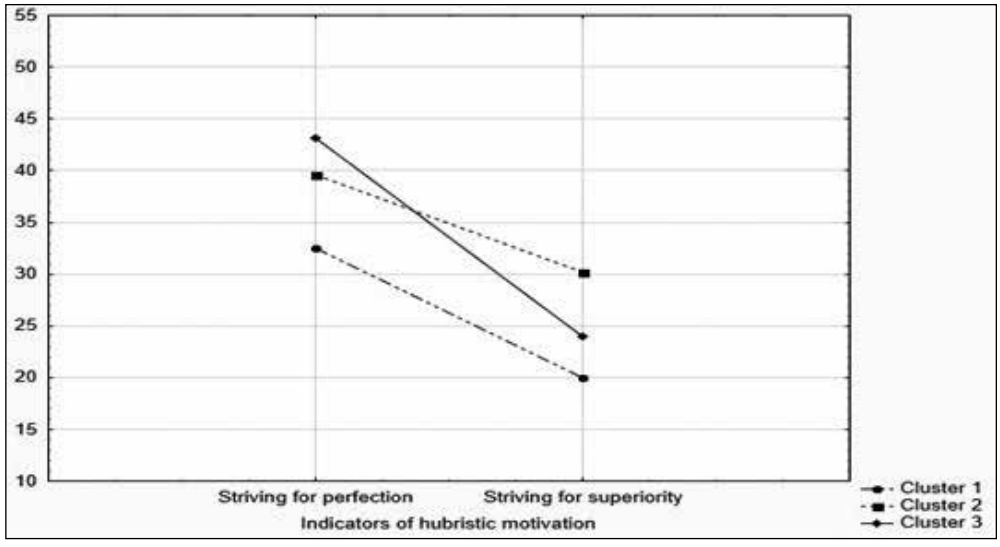

Fig. 1. Cluster profiles of hubristic motivation of adolescent athletes

According to the obtained cluster profiles, three groups of subjects have been identified: cluster 1: the mean indicators of striving for perfection and low indicators of striving for superiority – "Lower level of hubristic motivation" (n = 34, 29,3% of sampling); cluster 2: the mean indicators of striving for perfection and high indicators of striving for superiority – "Dominant striving for superiority" (n = 45, 38,8% of sampling); cluster 3: high indicators of striving for perfection and mean indicators of striving for superiority – "Dominant striving for perfection" (n = 37, 31,9% of sampling). The findings of the study on psychoemotional states in the sports activities of adolescent athletes with different typological profiles of hubristic motivation are presented in Table II.

It has been found that there are significant differences in the indicator of active-passive attitude to life situations (t = 3,03; p<0,005) between groups of adolescent athletes with a dominant striving for perfection and lower level of hubristic motivation. Significant differences have been found in the vigor-despair indicator (t = 2,71; p<0,01), between groups of adolescent athletes with dominant striving for perfection and lower level of hubristic motivation.

It has been established that adolescent athletes with dominant striving for perfection in sports were characterized by a higher level of activity in a situation of quarantine restrictions, compared to peers with lower level of hubristic motivation. Although activity is a relatively unstable characteristic, in this case the active attitude to

life situations in adolescent athletes, striving for perfection, was due to the direction of their motivation. Adolescents with lower level of hubristic motivation did not have such a stimulus. Adolescent athletes with dominant striving for perfection during the confinement period were more vigorous compared to adolescents with lower level of hubristic motivation. In stressful situations the latter are more prone to despair, they are more characterized by low mood, frustration with the course of events, a blurred sense of the future. Adolescent athletes with a dominant striving for superiority are more characterized by a cheerful mood, hypermotivity, a strong intention to act, which allows them to more easily overcome a period of quarantine restrictions.

The findings of the study of the choice of coping strategies by the adolescent athletes with different typological profiles of hubristic motivation are presented in Table III.

Apparently, there are significant differences in indicators of problem solving ($t = 4,12$; $p < 0,0001$) and the search for social support ($t = 3,29$; $p<0,005$) between groups of adolescent athletes with dominant striving for perfection and with lower level of hubristic motivation. The significant difference in indicators of problem solving ($t = 2,35$; $p<0,05$), search for social support ($t = 2,81$; $p<0,01$) between the groups of adolescent athletes with dominant striving for superiority and lower level of hubristic motivation has been established. There was also a significant difference in the indicator of problem solving ($t = 2,40$; $p<0,05$) between the groups with dominant striving for perfection and dominant striving for superiority.

The analysis of the differences in the three groups of adolescent athletes with different typological profiles of hubristic motivation revealed that adolescent athletes striving for perfection used such productive strategies as problem solving and seeking social support more often to reduce the risk of stressors during quarantine restrictions. The use of the above strategies enables finding solutions, expressing own active position, gaining social support, which helps to overcome confusion, to cope with anxiety in situations of uncertainty. On the contrary, adolescent athletes with lower level of hubristic motivation used these strategies much less frequently, which may worsen the course of adaptation during quarantine dynamic changes. Noteworthy, adolescent athletes with striving for superiority used these strategies less often compared to adolescents, striving for perfection, though more often than adolescents with lower level of hubristic motivation.

DISCUSSION

The study of the psychoemotional states of adolescent athletes in the period of quarantine restrictions has established the features of emotional distress of girls and boys, differences in the choice of coping strategies. Female athletes aged 13-14 had a higher level of anxiety and were more emotionally unstable compared to male athletes of the same age. Previous studies have shown the presence of distress in student youth, who are more adherent to preventive behavior during the Covid-19 pandemic [11]. The researchers highlight that respondents showed depression, frustration, anxiety, and more pronounced emotional instability. The distress in women is more acute. According to sociological research, there are differences in the emotional states of girls and boys. The level of anxiety in girls aged 15-18 years is higher than in boys of the same age [12]. It has been found that males are more even-tempered, they have a higher ability to control emotions and have more adequate reactions in extreme situations [13].

The established differences in the choice of coping strategies between adolescent athletes of different gender can be explained by the peculiarities of their psychoemotional states. Emotional instability of the female athletes determines the choice of strategy of avoidance, while the male athletes make the choice in favor of problem-solving behavior. The resulting data are partially confirmed by the findings of previous researchers, who emphasize that most of the resources the young men spend on constructive problem solving and success, whereas for girls it is more typical to seek social support. It should be noted that the above study was conducted on the sample of respondents who are not athletes and aged 18 to 23 years [14].

Other data obtained during our research are not correlated with the results of previous studies due to the lack of similar scientific research.

CONCLUSIONS

Thus, in the period of quarantine restrictions, the psychoemotional states of adolescent athletes are characterized by a number of features. The study revealed gender differences. Female athletes are more tending to emotional distress and mood swings compared to their male peers. To reduce the impact of stressors under quarantine, female athletes tend to choose the unproductive strategy to avoid problems, while male athletes are more likely to use such a productive coping strategy as problem solving. The psychoemotional state of adolescent athletes of individual sports is more stable compared to adolescent athletes of team sports. Restrictions on group interaction during the quarantine period made the training of sports teams difficult, which affected the state of the athletes. The dominant striving for perfection in the profile of humoristic motivation of adolescent athletes helps to maintain resilience and an active attitude to life situations, has a positive effect on the choice of behavioral strategies. Adolescent athletes, who are mostly striving for perfection, focus their efforts on problem solving and / or seeking social support during quarantine restrictions.

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ORIGINAL ARTICLE

STRUCTURAL AND FUNCTIONAL FEATURES OF PERIPHERAL BLOOD ERYTHROCYTES AND ANTIOXIDANT PROTECTION ENZYMES IN PATIENTS WITH MULTIPLE SCLEROSIS

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ABSTRACT

The aim: The aim of this work is to study the structural and functional features of peripheral blood erythrocytes and antioxidant defense enzymes in patients with multiple sclerosis, depending on its clinical forms and the stage of development of the pathological process.

Materials and methods: The state of the processes of lipid peroxidation and the antioxidant defense system in 62 patients with multiple sclerosis was studied. The influence of these processes on the clinical manifestations of multiple sclerosis is shown.

Results: It has been shown that in multiple sclerosis, the erythrocyte superoxide dismutase / catalase enzyme system is characterized by the phenomenon of cross-regulation of activity, which is reflected in a negative correlation between enzyme activities and is associated with the appearance of irreversibly altered forms of erythrocytes in the general bloodstream.

Conclusions: Based on the analysis of the dynamics of laboratory parameters, the pathogenetic significance of membrane-destabilizing processes in peripheral blood erythrocytes of patients with multiple sclerosis was proved.

KEY WORDS: multiple sclerosis, erythrocytes, antioxidant defense enzymes

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INTRODUCTION

The steady increase in the prevalence and severe socio-economic consequences of multiple sclerosis (MS) necessitate further analysis of this pathology, in particular, the study of its pathogenesis, as a key to finding promising methods of pathogenetic therapy. In the pathochemical complex forming in MS, the central place is given to the disintegration of metabolism, activation of the processes peroxidation of lipid (POL) and suppression of the antioxidant defense system (ADS) [1-4]. The relationship between disorders in the immune system and the accumulation of lipid peroxide products in the body has been established. Excessive content of POL products in the body serves as one of the factors in the formation of autoimmune responses [1-3]. Based on the works of individual authors [5], the study of the severity of structural changes in cell membranes is promising from the point of view of their relationship with the severity and prognosis of the disease, while the state of ADS can determine the degree of recovery of neurological deficit. To reveal the pathogenetic mechanisms of the formation of damage at the tissue and cellular level in MS, it is of considerable interest to study the role of the erythron system as an important link in providing compensatory-adaptive reactions of the whole organism. Erythrocytes of peripheral blood (EPB), in close contact with all tissues and entering into morpho-functional relationships with them, their own qualitative and quantitative restructuring reflect the physiological and pathological changes occurring in the body, thereby causing the so-called exvisit (displayed) reactions that are used in as prognostic markers in a number of chronic diseases [6]. The multifunctional role of EPB in the mechanisms of adaptation and compensation under conditions of hypoxia, during the implementation of gas transport processes and other vital functions explains the high information content of the results of studying structural and functional changes in these cells. At the same time, an insufficiently studied aspect of hypoxia is the enzymatic regulation of the processes of formation and destruction of hydrogen peroxide in EPB. Taking into account the data on the direct participation of reactive oxygen species (ROS) (O2, H2O2) and antioxidant defense enzymes superoxide dismutase (SOD) and catalase (CAT) in the processes of hemoglobin oxygenation [7], It is of interest to study the nature of changes in the activities of these enzymes aimed at increasing the structural and functional usefulness of EPB, which is necessary for adequate oxygen transport in MS.

THE AIM

The aim of this work is to study the structural and functional features of peripheral blood erythrocytes and antioxidant defense enzymes in patients with multiple

sclerosis, depending on its clinical forms and the stage of development of the pathological process.

MATERIALS AND METHODS

We examined 62 patients with a reliable diagnosis of multiple sclerosis according to McDonald's criteria (2017), the average age of patients was 40.4 ± 0.87 years, EPB studies were carried out during a hospital stay (for 14 days) and during an exacerbation of the disease (throughout 2019-2020 yers.)

Patient groups were formed in accordance with modern guidelines and the type of MS development. All patients were divided into three groups: patients I group. - with relapsing multiple sclerosis (RMS), patients II gr. - with secondary progressive multiple sclerosis (SPMS) and patients of III group - with primary - progressive multiple sclerosis (PPMS). All MS patients had no comorbidities. The control group (CG) consisted of 20 practically healthy people, comparable in age.

The degree of compensation of the disease in RMS was conditionally divided as follows: decompensated - 2 and more exacerbations in the last year, subcompensated - 1 exacerbation and compensated - 0 exacerbations. Studies in RMS were carried out: 1) during exacerbation and 2) during remission.

The severity of the neurological deficit ranged from 7 to 9 points on the EDSS scale in group I. from 4 to 6 points in II gr. i was 1-3 points in III group.

Special research methods are presented:

By studying the content of the products of free radical oxidation of lipids in EPB membranes: diene conjugates (DC) of polyunsaturated fatty acids of phospholipids and Schiff bases (SHB) [2, 7].

Evaluation of the level of antioxidant protection (content [8], activity of superoxide dismutase (SOD) - an enzyme of antiradical defense, catalase (CT) - an enzyme of ADS, glucose-6-phosphate dehydrogenase (G-6-FDHG) [9].

Determination of phospholipase activity [9]. The surface architectonics of the EPB was studied using scanning electron microscopy (SEM) (electron microscope „JEOL-25A-T3225"; Japan) with the preparation of samples by the method of GI Kozinets et al. [10].

Statistical analysis was performed using the standard SAS 8.0 software package (SAS Inc., USA). The t and $\chi 2$ criteria were used. The results were considered reliable at $p < 0.05$. Some of the studies were carried out on a hemoanalyzer (Lab Analyt30000Plus (Finland)). Determination of the microelement composition of EPA (nitrogen, calcium, magnesium) was carried out using energy-dispersive X-ray analysis on an attachment for microanalysis „EDAR" to SEM „REMMA-202E" (Sumy, Ukraine).

Determination of the index of erythrocyte deformability (IDE) by the method of C. Tannert, V. Lux (1981) modified by Z.D. Fedorova, M.O. Kotovschikova and saturation of blood with oxygen using pulse oximetry device „Jziki-Fingertip oximeter".

RESULTS

The state of membrane-destabilizing processes is characterized by various criteria. This is, first of all, the level of functional activity of endogenous phospholipases and the dynamics of lipid peroxide accumulation. As follows from our results, the severity of membrane-destabilizing processes increases from the minimum values in patients with I group. with the most favorable relapsing form of MS (RMS) to the maximum level in II gr. with secondary progressive MS (SPMS) and III gr. with primary progressive MS (PPMS), which is manifested by an increase in the width of the hemogram and its change from the normal, unimodal type of hemogram (Fig. 1 B) on the bipolar type of EPA distribution over their volume (Fig. 1 A), and a significant shift of the distribution peak to the left (Fig. 1 B).

In patients II and III gr. found changes not only in the form of EPA, but also in their chemical composition, which was most clearly manifested in patients III group. As MS progressed, erythrocytes from biconcave discs turned into spherocytes, and pronounced microrelief irregularities appeared on them. The ratio of spherocytes to discocytes in patients who have not had a single exacerbation over the past year, which we conditionally assumed as a stage of compensation. was 1: 7, in the stage subcompensation (1 exacerbation in the last year) was 1: 4, in the stage of decompensation - 1: 9, it should be noted that such a gradation is conditional and adopted by the authors of the article for a more accurate characterization of patients (in practically healthy individuals from the CG and of the same age, this ratio is 1:60) (fig. 2). In such conditions, patients III gr. with an increase in the number of spherocytes in comparison with CG people, the amount of nitrogen, a well-known basic chemical element of living proteins, decreases statistically significantly (by 3 times; $p < 0.05$) (Fig. 2, Table 2), which allows you to identify the microanalyzer attachment „EDAR" to a scanning electron microscope. It is known that proteins in the EPB membrane are located mainly on its inner side and form a network of filaments that support the biconcave shape of the EPB [11].

Thus, the loss of the disc-shaped form of EPB is explained by a deficiency of free nitrogen and an increase in the amount of nitric oxide, which is possible under conditions of nitrozoline stress and the breakdown of protein structures of the cytoplasmic membrane of the EPB. Protein breakdown was exacerbated by exacerbations of MS.

In the EPB of patients II and III gr. compared with EPA of practically healthy CG people of the same age, a significant ($p < 0.05$) increase in the concentration of calcium (1.5-2 times), magnesium (2 times) and aluminum (3 times) was found (Fig. 3). This should be interpreted as a sign of the development of the calcium paradox with a decrease in the amount of free nitrogen forms and an increase in nitric oxide upon activation of LPO [12], which is the reason for the violation of the permeability of cell membranes and the approach of EPB to hemolysis.

The aluminum, most likely, was not in the erythrocytes themselves, but was "knocked out" by an electron beam from the substrate of the blood SEM preparation. This closely correlates ($r = 0.93$; $p < 0.001$) with the appearance of cells with an irreversibly changed shape among the erythrocyte population (Fig. 4).

The hemogram of the EPB size distribution is asymmetric and multipolar due to an increase in the number of varying classes of small-sized (left) wing of cellular elements (Fig. 5).

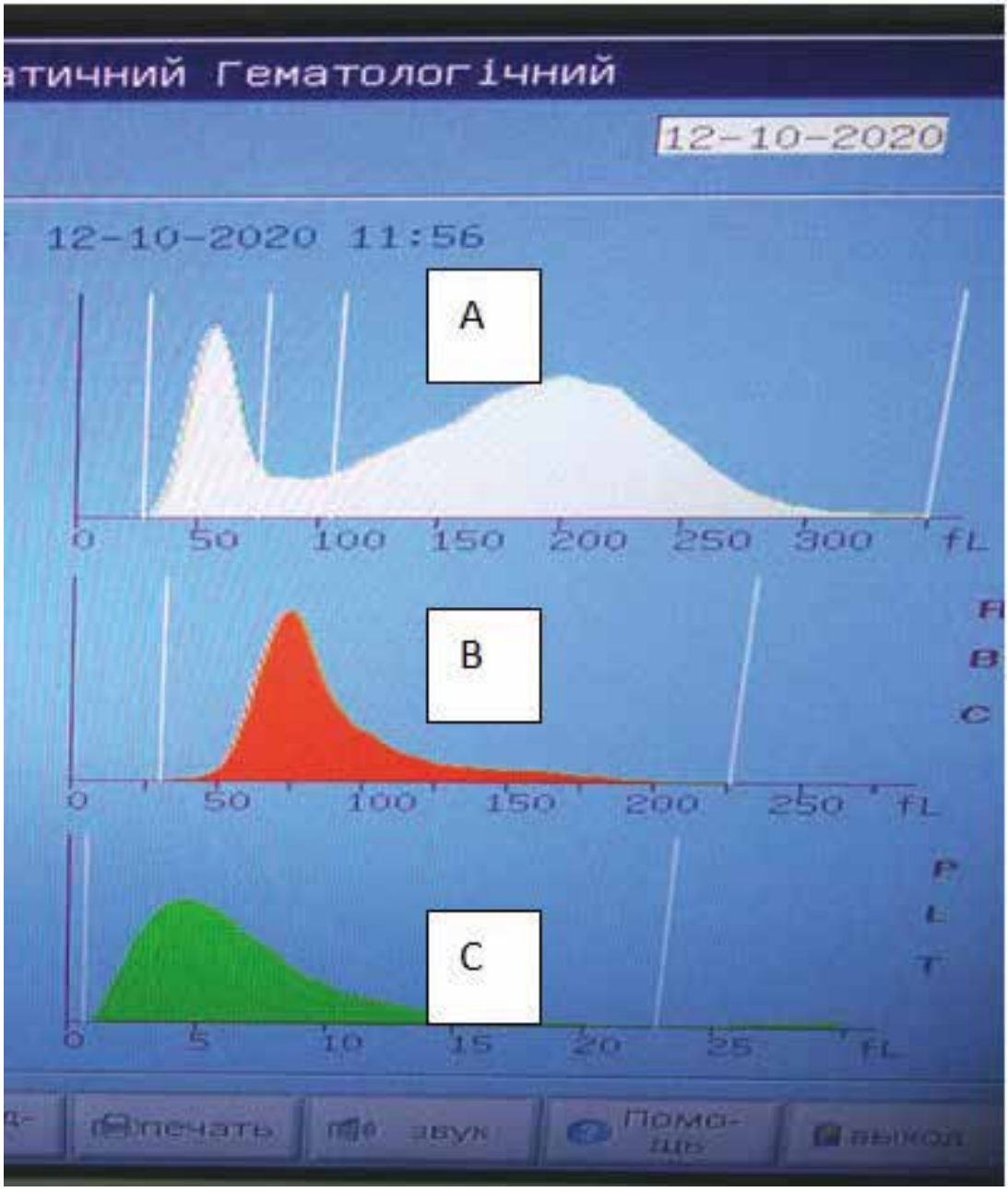

Fig. 1. Hemograms of EPA distribution by the mean volume (FL) of the patient I gr. (C), IIgr. (A), and the patient CG (B) and with a sharp shift to the left (C)

At the same time, the biochemical parameters of EPA change (Table I), which is especially revealed when comparing the indicators obtained during the exacerbation period and during the period of remission of MS (Table II).

Thus, in MS patients, regardless of the form of the disease, a multiple increase in the content of LPO products (diene conjugates (DC) and Schiff bases (III)), as well as the activity of phospholipase-α2 (PLA-α2) relative to CG ($p < 0.05$) was revealed. Moreover, as the disease progresses, the content of POL products (DC, IIIO) naturally increases ($p < 0.05$) and closely correlates ($r = 0.93$; $p < 0.05$) with an increase in the amount of EPA of an irreversibly altered form (Fig. 6 -8), which can serve as a prognostically unfavorable indicator in patients with MS, since the formation of acanthocytes and echinocytes leads to a sharp decrease in the area of effective metabolism with the EPB membrane, due to the very small area of their contact with the endothelial cells of the micro microcirculatory net work (MCNW) working organs, only a small surface at the tops of the properties of blood [13]. These outgrowths often injure the surface of MCB endotheliocytes (the surface glycocalyx is erased, when the rigid microrelief and structure of echinocytes acquire the properties of a kind of pipeline brush in relation to a very vulnerable surface of endotheliocytes), which is the cause of microthrombotisation of blood exchange and disruption of blood supply substances in the microenvironment of the hemocapillaries damaged in this way.

Thus, there is a pattern manifested by an increase in the activity of phospholipase-α2 in comparison with similar indicators in healthy CG people and it can be argued that it is the high activity of phospholipase-α2(PLA-α2) that pro-

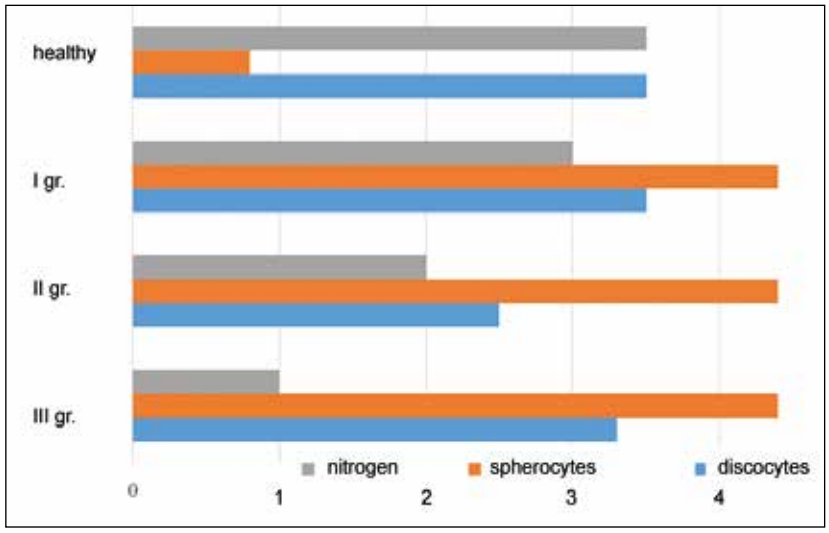

Fig. 2. The quantitative ratio of different forms of EPB and the concentration of nitrogen in erythrocytes of patients of different groups with MS

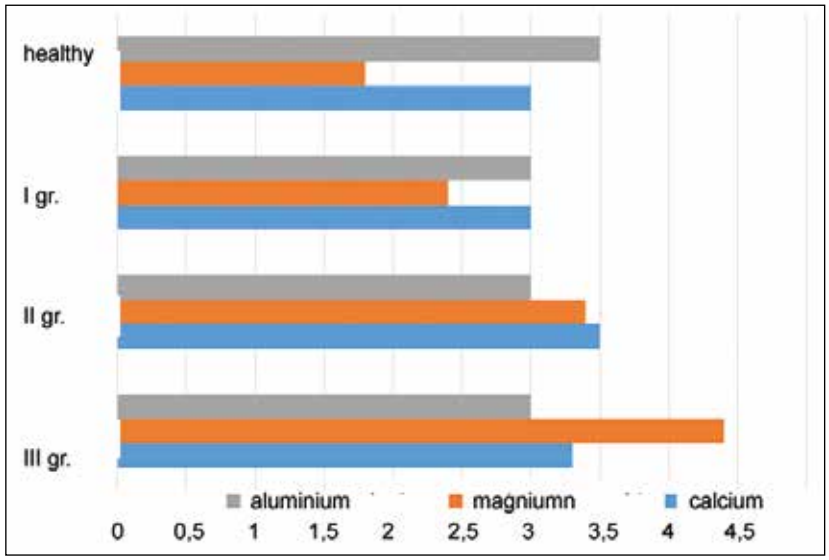

Fig. 3. Concentration of individual microelements in EPA in patients of different groups with MS

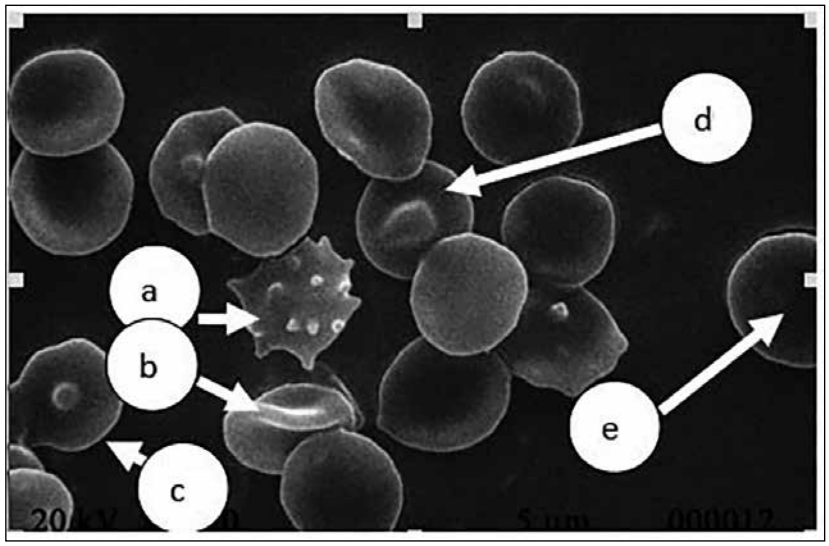

Fig. 4. Acanthocyte (a), erythrocyte with a ridge (b), erythrocyte with one outgrowth (c), erythrocyte with a dome (d), spherocyte (e) in patients II and III gr. Method: scanning electron microscopy

vides a less favorable course of MS in patients with group I. This is also evidenced by the quantitative characteristics of the activity of endogenous phospholipases and the processes of peroxidation of membrane lipids, depending on the stage of the disease. The data are presented in table II.

It was found that the content of lipid peroxides and the activity of phospholipase-α2 in EPB membranes were statistically significantly ($p < 0.05$) increased in patients with MS, regardless of the stage of the disease, and during the period of clinical remission, when these indicators did not correspond

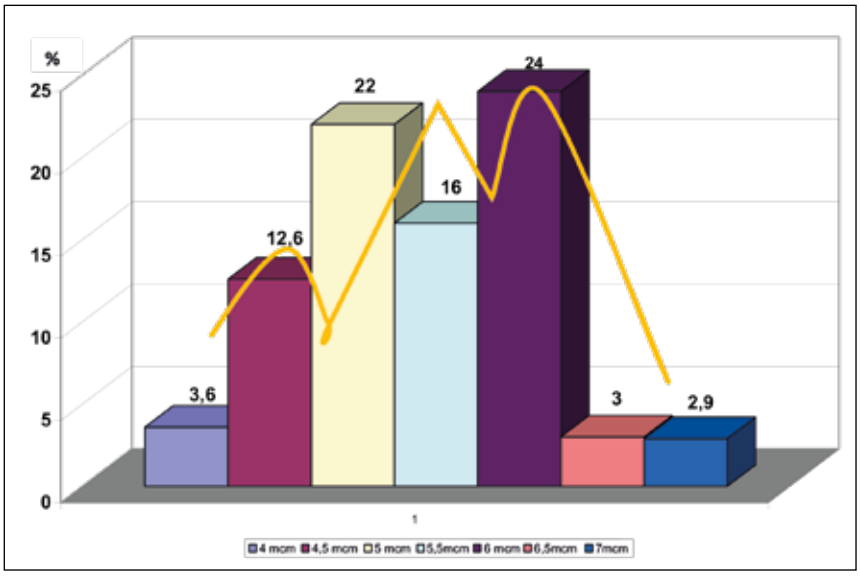

Fig. 5. Distribution of erythrocytes of patients of group III by maximum diameters in separate parametric groups.

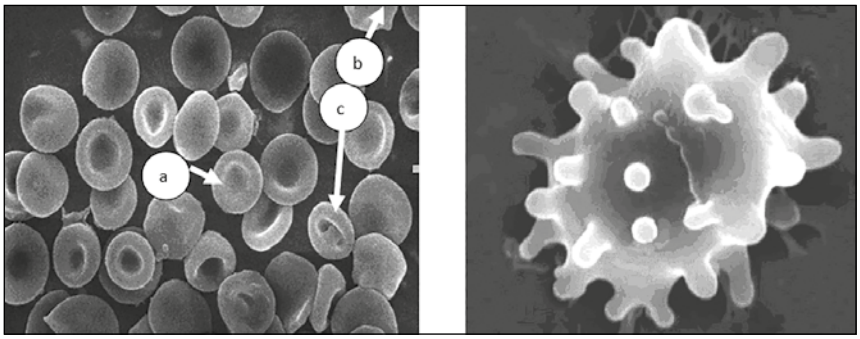

Fig. 6. Different forms of EPA (A): discocyte (a), erythrocyte with one outgrowth (b), erythrocyte with a ridge (c), and irreversibly changed form of EPB (B) – echinocyte in a patient of III gr with MS.

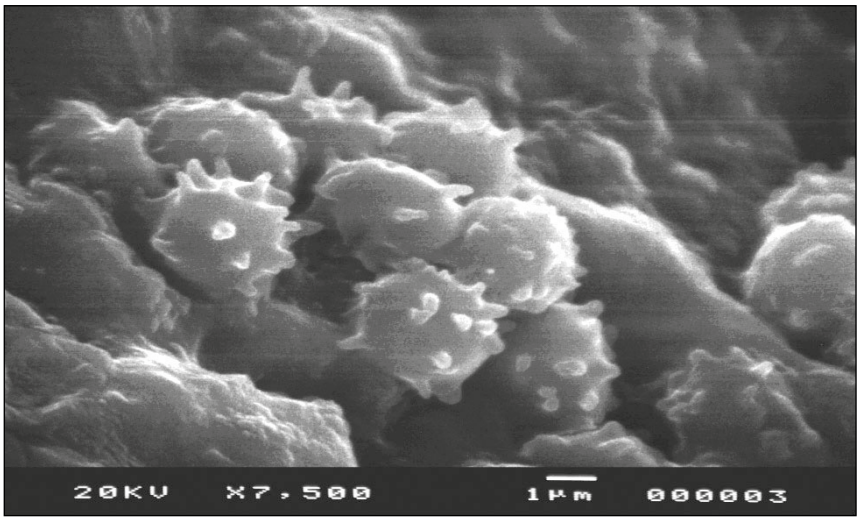

Fig. 7. Erythrocytes with multiple outgrowths – echinocytes in patient III, gr. with MS.

to the normative parameters. At the same time, we can assert that these changes are pathogenetically significant in the event of an exacerbation of MS (the difference between the analyzed parameters during exacerbation and remission of the disease is statistically significant, $p < 0.05$). Significant differences in the activity of PLA-α2, depending on the stage of MS, suggest that this indicator has a pathogenetic significance, increasing during exacerbation and decreasing during remission of the disease. The state of the antioxidant defense system in the body is also one of the most important mechanisms regulating POL activity and, indirectly, the functional significance of endogenous phospholipases. Presented in table. 3 results may indicate that with an unfavorable variant of MS (SPMS and PPMS) in patients with II and III gr. there is a breakdown of the compensatory capabilities of the studied ADS links, while in patients of group I with RRMS there were no significant differences from the CG.

Thus, it can be argued that the failure of the antioxidant defense system is an additional factor leading to the activation of POL processes and, ultimately, to a change in the functional properties of cell membranes in EPB in patients with group I.

In EPB CG, there were no significant changes in the activities of superoxide dismutase SOD, catalase and 6-FDG.

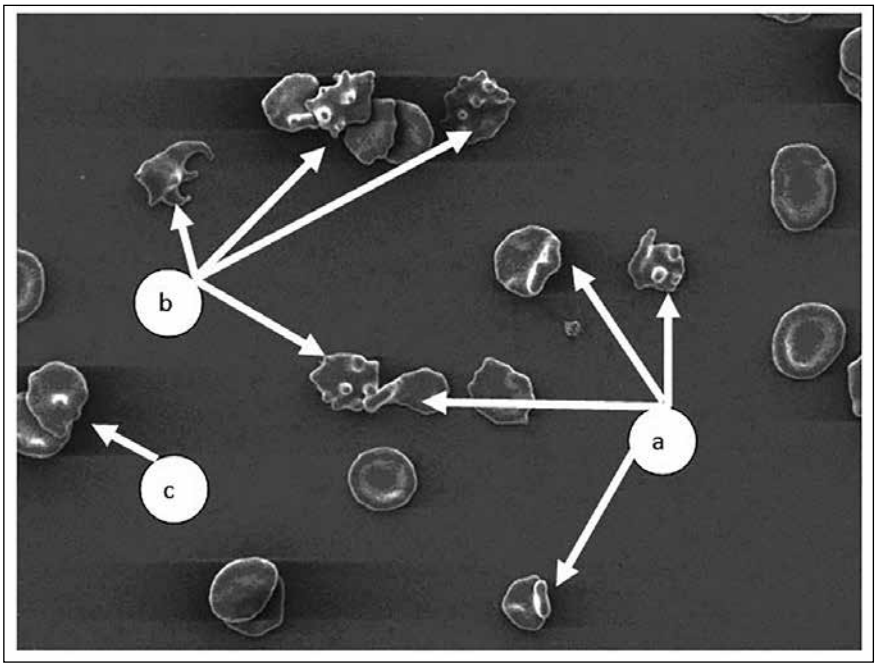

Fig. 8. The population of EPB of the patient III gr. in MS: erythrocyte with a ridge (a), acanthocyte (b), erythrocyte with one outgrowth (c).

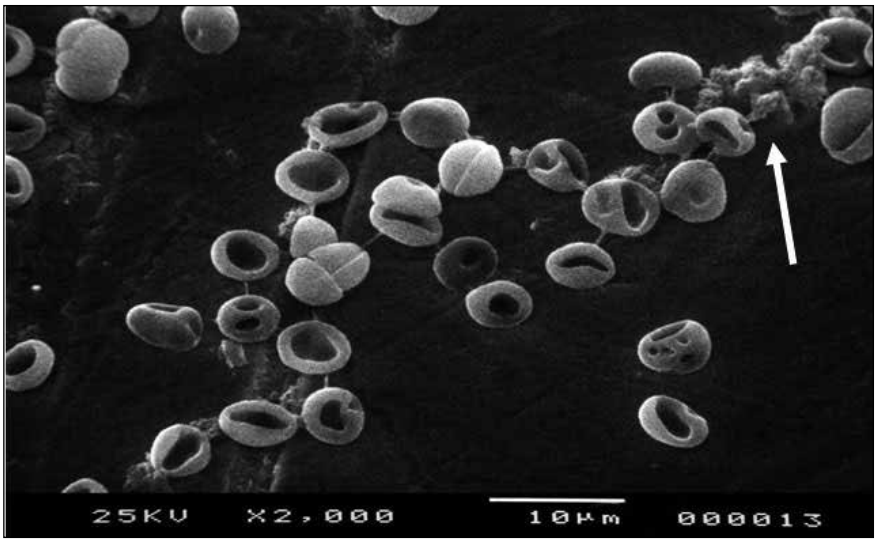

Fig. 9. Irreversibly altered forms and the formation of „shadows" of erythrocytes (shown by an arrow) in a patient III gr. with MS.

The average values of the studied enzymes were: SOD - 45.3 ± 2.9 0.25 ± 0.019 μmol / min / mg Hb, catalase - 3.9 ± 0.1 0.2 ± 0.05 μmol / min / mg Hb. and G-6-FDG - 662.9 ± 29.9. However, in patients with Igr. there is a decrease in activity and even a deficiency of G-6-FDG is observed, which determines a decrease in the energy reserves of cells, which, according to some authors [14], s the cause of the pathological conformation of EPB with the formation of „shadows" of erythrocytes (Fig. 9), which have an increased negatively charged surface potential in relation to the normal disc-shaped forms of EPB. It is known that the presence of „shadows" of erythrocytes in the general blood flow leads to the development of acute renal failure and increased mortality among patients with various and not only neurological pathologies.

Changes in the activities of catalase and SOD in EPB, depending on the group of patients in the dynamics of MS, have an oscillatory character. The growth of catalase activity in all cases occurs against the background of a significant decrease in SOD activity relative to catalase (CAT). Many enzymes, including SOD and CAT, are characterized by the phenomenon of cross-regulation of their activity [15]. For CAT, the superoxide radical anion is a negative effector, while H2O2 is a positive one, while for SOD it is vice versa (Fig. 10).

DISCUSSION

Our data were confirmed in the works of G.N. Okuneva. et al. [16]. The spherulation of EPB found by us and the accumulation of chemical elements inside them probably precede hemolysis, i.e. destruction of erythrocyte membranes with the release of hemoglobin into the blood. So, according to I.I. Gitelzon and I.A. Tereskova [17] with hemolysis of EPB go through a number of stages: 1) prehemolytic stage, characterized by spherulation of erythrocytes; 2) the stage of osmotic hemoglo-

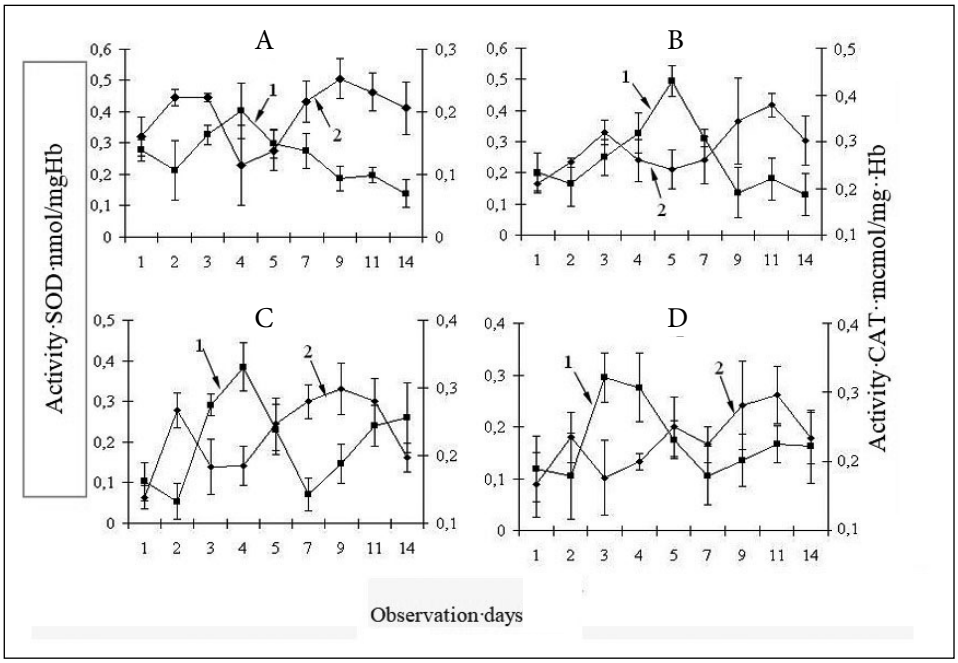

Fig. 10. Changes in the activities of SOD (1), catalase (2) in the dynamics of MS in peripheral blood erythrocytes of patients I gr. (A), gr. II (B). III gr. (C) and during an exacerbation (D).

binolysis, accompanied by swelling of the erythrocyte beyond the critical volumetric size (which in our case determines the expansion of the hemogram of the EPB volume distribution) with a change or damage to the cell surface and the release of most of the hemoglobin into plasma; 3) the stage of chemical hemoglobinolysis, characterized by a change in the chemical composition of the cell, and, accordingly, by a change in the electrochemical and colloidal-osmotic properties of EPB with complete elimination of hemoglobin; 4) the stage of complete destruction of cellular structures [17]. It would be logical to expect the development of anemia, predominantly of a hemolytic nature, also in patients with MS. Indeed, the anemia syndrome in the patients we examined was encountered in 6.7 - 11.5% of cases with exacerbation of MS and in 33.3% of cases with a decrease in blood oxygenation (up to 76-82% determined by pulse oximetry). According to the scientific literature, cardiovascular mortality in MS patients increases in proportion to the deviation of the hematocrit index, both towards anemia and towards polycythemia. So, back in 2007 W.-C. Wu et al. [18], found a 1.6% increase in mortality associated with every percentage of hematocrit abnormalities in either direction. Therefore, in the chronic course of MS and against the background of membrane-destabilizing processes in the EPB a more unfavorable prognosis for life can be expected than in the case of the usual chronic form of MS with a mildly pronounced neurological deficit, which developed against the background of an exacerbated stage of the disease, combined with destructive changes in EPB. Indeed, firstly, the compensatory capabilities of EPB are much less during the development of POL processes.

Due to the smaller thickness of the EPB membrane, and, secondly, the degree of hypoxia in the presence of metabolic pathology increases significantly. Among the MS patients we examined, lethality in the case of exacerbations of the neurologically deficient type was 0.93%, and for the vegetatively deficient type - 28.6%, which confirms our thesis of a pronounced exvisit reaction EPB in MS. It is known that many diseases, including immune-neurological ones, are characterized by the development of oxidative stress as a result of a violation of the prooxidant-antioxidant balance and an increased course of free-radical reactions with the participation of reactive oxygen species [2, 4, 5]. At the same time, in recent years, a lot of data have accumulated, indicating a close relationship between the production of free radicals of oxygen and nitric oxide [7]. There is evidence that nitric oxide (NO) is one of the universal regulators of the physiological functions of the body with a fairly wide spectrum of biological action [13]. Thus, it is known that at optimal concentrations, NO improves the endothelial function of peripheral vessels, has a positive effect on the activity of individual protein kinases, is able to inhibit caspases, and inhibit the induction of apoptosis [13, 14]. However, the synthesis of nitric oxide in high concentrations exceeding the permissible stationary level, may be the cause of the development of nitrosative stress caused by the formation of reactive nitrogen species (RNS), primarily peroxynitrite and the product of its degradation, nitrogen dioxide [19]. One of the markers of nitrosative stress the formation of low molecular weight and high molecular weight nitrosothiols, in particular, products of protein nitrosylation, is considered [20]. Previously, it was shown that in ischemic heart disease (IHD) in the EPB, the reactions of lipid peroxidation and oxidative modification of proteins are enhanced [21]. Considering this and the fact that in a number of diseases erythrocytes are involved in the pathological process [22], it was of interest to study individual indicators of the nitric oxide synthesis system and nitrosylation processes in erythrocytes of MS patients, which will be the goal of a future study. Changes in the morpho-functional state of EPB in the form of their spherulation and the development of chemical imbalance (loss of nitrogen by cells and accumulation of calcium and other chemical elements inside them) can be considered signs of an unfavorable prognosis for the life of patients with chronic neurological insufficiency in MS, and the SEM method of blood samples – is diagnostically significant in these patients.

Table I. Indicators of PLA–α2, activity and the content of LPO products in EPB of MS patients, depending on the form of the disease (M±m)

Indicators	Healthy n=20	MS patient group, n=60		
		RMS (n=20) I gr.	SPMS (n=20) IIgr.	PPMS (n=20) III gr.
PLA –α2, % hemolysis	4,44±0,22	15,21±1,1 *	27,05±1,3 */ **	25,93±1,33 */ **
DC, nmol/ml	48,43±3,5	109,11±3,7 *	180,33±5,1 */ **	170,11±7,93 */ ***
SHB, c.u.	18,3±1,02	21,73±5,1 *	30,11±2,33 */ **	33,11±2,53 */ **
IDE	2,03±0,005	1,44±0,002	1,39±0,003	1,08±0,002***

Note: IDE – is the index of erythrocyte deformability;
* – reliability of statistical differences between indicators of healthy and sick ($p < 0.05$);
** –the reliability of statistical differences between the indicators of RMS patients and indicators in the other two groups of patients with MS ($p < 0.05$);
*** – reliability of statistical differences between indicators of patients with SPMS and PPMS ($p < 0.05$).

Table II. Indicators of the activity of antioxidant defense enzymes and the content of α-tocopherol in the EPB of patients with MS, depending on the form of the disease (M ± m)

Indicators	Healthy.n=20	MS patients, n=60		
		RMS (n=20)	SPMS (n=20)	PPMS (n=20)
SOD, % braking	45,33±2,91	48,36±1,92	28,54±1,22*/ **	21,14±0,88*/ **/ ***
Catalase, mol min. ml	3,95±0,11	3,19±0,11	2,01±0,09*/ **	1,87±0,11*/ **
G-6FDG, ml / ml	662,93±29,91	607,13±26,71	417,8±21,12*/ **	358,44±23,82*/ **/ ***
α-tocopherol, nmol / ml	5,84±0,28	5,03±0,11	3,67±0,11*/ **	3,24±0,22*/ **

Note:* – reliability of statistical differences between indicators of healthy and patients with MS ($p < 0.05$);
** – reliability of statistical differences between indicators of RMS patients and indicators in two other groups of patients with MS ($p < 0.05$);
*** – reliability of statistical differences between indicators of patients with SPMS and PPMS ($p < 0.05$).

Such a multidirectional change in the activities of these two enzymes, according to the scientific literature, is characteristic of hypoxia and energetic insufficiency of cellular metabolism [7]; under conditions when activation of membrane-destabilizing processes underlying the conformational transformation of EPB is observed. Apparently, this pattern is also inherent in patients with MS.

Statistical analysis of the experimental data obtained showed a significant negative correlation between the activities of SOD and erythrocyte CAT in patients of all studied groups. In CG patients, a high (+0.86) positive correlation between the activities of these enzymes was recorded.

There is an opinion that the reduction of hydrogen peroxide can serve as an additional source of molecular oxygen [23].

Catalase, performing an antioxidant function, compensatory increases the coefficient of useful use of exogenous oxygen for energy purposes due to the partial return to the metabolic chains of oxidative phosphorylation of that molecular oxygen, which is reduced in the body through the one-electron path.

The destruction of myelin in MS leads to the release of proteolytic enzymes and other aggressive agents from neurolemmocytes, which irreversibly changes the conformation of protein molecules of damaged cells, which are perceived by the immune system as „alien". For example, some authors [24] found histological signs of myocardial dystrophy in rats already on the 7th day after the action of stress factors. The authors note that a sharp decrease in oxygen supply to cardiomyocytes occurs both as a result of the development of edema of the vascular wall and perivascular space, and as a result of direct destruction of the vessels of the microvasculature, observed from the first days of the stress reaction. It was shown [25] that MS causes changes in the structure of the mitochondrial population in neurons of different types. Morphofunctional changes in the tissues of the thymus [26] and kidneys [27] have been described at different periods of exposure to stress factors. with aging, hypoxia[28], which allows us to consider the response of the body of patients with MS to the pathological effect of the basic myelin protein as specific and due to the discrepancy with the increasing energy demand in MS and the possibilities of energy generating systems for its compensation. A number of researchers detect in the blood of persons with MS antigens of the liver, kidneys, heart, muscle, nervous and connective tissues, as well as high titers of antibodies to these antigens [26]. At the same time, inflammatory processes occur in their bodies, primarily in the nervous tissue and find regular manifestations in the form of conformational changes in EPB, which we propose to use for diagnostic and prognostic purposes.

The results of the study indicate that a significant place in the pathogenesis of MS belongs to the activation of enzymatic and free radical oxidation of cell membrane lipids under conditions of complete or partial failure of antioxidant defense mechanisms. This results in significant changes in the structure of the EPB cell membranes and their function. The changes identified in the EPB model are basic, correlate with the severity of clinical manifestations of MS, which means that they largely determine what can be used to determine not only the severity of MS, but also to predict its course, as well as to control the quality of therapeutic measures in such patients.

Thus, POL leads to the development of a „calcium paradox" in the EPB, which determines a low cellular energy potential

not only of the EPB themselves, but also of neurolemmocytes, which is the reason for their low regenerative capacity, which is an unfavorable indicator in prognostic terms. This explains why the appearance of a large number of irreversible EPB and an increase in the width of the distribution of erythrocytes in the cell volume is a prognostically important and unfavorable sign in patients with MS.

Because Jacques Lucien Monod, the Nobel laureate in the field of membranopathy, stated: „What is true for *E. coli* will be true for an elephant as well", and in our case: „What is observed in erythrocytes will be observed in the whole organism".

CONCLUSIONS

1. The different course of multiple sclerosis is accompanied by a change in the activity of the enzymes superoxide dismutase and catalase, which is associated with the activation of the formation of reactive oxygen species and occurs against the background of a 2-fold decrease ($p < 0.05$) in the value of the deformability index of erythrocytes and the concentration of nitrogen in erythrocytes of peripheral blood, which may be one of the pathogenetic factors in the progression of multiple sclerosis.
2. It has been shown that in multiple sclerosis, the erythrocyte superoxide dismutase / catalase enzyme system is characterized by the phenomenon of cross-regulation of activity, which is reflected in a negative correlation between enzyme activities and is associated with the appearance of irreversibly altered forms of erythrocytes in the general bloodstream.
3. An increase in the ratio of superoxide dismutase / catalase above the level of the control group is observed in patients II gr. This fact is due to a drop in catalase activity and can be regarded as a state of hypoxia.
4. Exacerbation of multiple sclerosis in RMS is accompanied by a decrease in the activities of both superoxide dismutase and catalase and a decrease in their ratio to a level below 1. This is closely correlated ($r = 0.93$; $p < 0.05$) with conformational changes in erythrocytes, which should be used in diagnostic -prognostic purposes in multiple sclerosis.
5. An increase in catalase activity with a constant activity of superoxide dismutase is possibly associated with the activation of peroxisomal enzymes or other parallel processes that require an increase in the activity of catalase in the cell.

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ORIGINAL ARTICLE

ASSOCIATIONS OF ENOS GLU298ASP (G894T) ENDOTHELIAL DYSFUNCTION GENE POLYMORPHISMS WITH METABOLIC DISORDERS IN PATHOLOGICAL PREGNANCY

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ABSTRACT

The aim: To determine the association between the Glu298Asp (G894T) polymorphisms of the eNOS gene with metabolic disorders in excessive gestational weight gain (GWG) pregnancy.

Materials and methods: 97 pregnant women in 9-12, and 37-39 weeks of gestation were examined. The recommended GWG was diagnosed in 33 (34.0 %), insufficient in 19 (19.6 %), and excessive in 45 (46.4 %) patients. Genetic variants of eNOS were analyzed by real-time polymerase chain reaction, lipid profile, and carbohydrate status were performed. The results were statistically analyzed using Statistica 6.0 program pack (StatSoft Inc., USA) and Microsoft Excel statistical analysis package.

Results: GWG in the group of patients with gene eNOS TT polymorphism was significantly higher (1.5-fold) compared to pregnant with GG – genotype ($p<0.05$). Pregnant, inherited the TT allele of the eNOS gene, associated with higher levels of hypertriglyceridemia, hypercholesterolemia, of lipoprotein low density, glycemia, hyperinsulinemia, and higher HOMA–IR compared to carriers of GG and GT alleles ($p<0.05$) in the third trimester, which is especially manifested in excessive GWG.

Conclusions: Decreased activity of the eNOS gene in the presence of pathological alleles is the initiator of impaired lipid and carbohydrate metabolisms and, as a consequence, excessive GWG. A high risk of excessive GWG was found in pregnant women with TT polymorphism Glu298Asp (G894T) (OR=4.52; 95%CI: 1.18-17.32; $p<0.05$) (frequency distribution of 73.7 %). Endothelial dysfunction is a pathogenetic link of excessive body weigh in pregnancy.

KEY WORDS: lipid profile, gestational weight gain, eNOS Glu298Asp (G894T) gene polymorphisms, carbohydrate status

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INTRODUCTION

The problem of genetic determinism of the risk of metabolic disorders during pregnancy has recently attracted an increasing attention of scientists. A topical issue is the study of the association between endothelial dysfunction and overweight in pregnant women in terms of predicting maternal and perinatal complications [1-3].

The leading link in endothelial dysfunction is the reduction of nitric oxide (NO) synthesis, which is a potent vasodilator. It is synthesised from L–arginine by nitric oxide synthase (NOS). In humans, NOS has three main isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). NO formation is regulated by altering the expression or activity of the eNOS enzyme, or by altering the activity of cofactors or endogenous inhibitors [4]. eNOS is an important angioprotective mediator and dysregulation of its synthesis is associated with an increased risk of cardiovascular diseases, including hypertension during pregnancy [5]. Therefore, the processes of regulation of NO synthesis, genes and polymorphism of genes encoding NO synthesis, or affecting the activity of enzymes NO synthases, are the primary candidates for the study of endothelial dysfunction.

eNOS gene is located on the long arm of human chromosome 7 (q35.1), and it contains 27 exons and 26 introns with a total length of 24 kb. There are several polymorphisms in NOS3 gene. Modern researches are focused on the study of three polymorphic gene variants: T786C eNOS gene promoter polymorphism, G894T (Glu298Asp) exon 7 polymorphism, 4a / b – 4 eNOS intron. The rs1799983 polymorphism (also known as G894T or Glu298Asp) is located in exon 7 of eNOS gene and formed by a transversion from guanine (G) to thymine (T). Accordingly, the 298th genetic code is changed from GAG to GAT, resulting in the replacement of glutamic acid residue with aspartic acid residue in the eNOS polypeptide. This polymorphism has been shown to significantly reduce the promoter activity of the eNOS gene and is associated with a decrease in the basal level of NO production [6]. It has been shown in the literature, that endothelium-dependent vasodilation in the presence of the 298Asp allele (894T) is damaged and this polymorphic variant is a predictor of hypertensive disorders and atherosclerosis [5, 7].

Nassereddine S et al. evaluated the potential association of SNV rs1799983 of the NOS3 gene with hypertension

susceptibility among a sample of Moroccan patients [8]. They proved that the SNV rs1799983 of the NOS3 is responsible for reduced NO synthesis and essential hypertension development. Their results also showed a positive correlation between rs1799983 NOS3 distribution and obesity risk factors (p=0.02).

Endothelial disorders are widely studied by scientists, also in overweight people. However, to date there is no single view on the development of endothelial dysfunction in this pathology. A number of metabolic and hemodynamic disorders are associated with overweight. There is no clear position among the authors as to the statement whether these conditions are a complication of high body mass index (BMI) or whether they are comorbidities, whose occurrence and progression are exacerbated by the presence of excessive body weight. Proponents of the first theory claim that endothelial dysfunction is secondary to existing insulin resistance, hyperglycemia, and dyslipidemia [9]. Hyperglycemia in endothelial cells promotes the activation of the enzyme protein kinase-C, which increases the permeability of endothelial cells to proteins and disrupts endothelium-dependent vascular relaxation. Hyperglycemia also activates peroxidation processes, the products of which inhibit the vasodilating function of the endothelium. Dyslipidemia increases the expression of adhesive molecules on the surface of endothelial cells. As a result, the endothelial cells membranes are gradually damaged, their architectonics is disturbed, the secretion of vasoconstrictor endothelin-1 is enhanced and the synthesis of enzymes, necessary for the secretion of vasodilators, is inhibited [1].

Supporters of the other hypothesis believe that endotheliopathy is not a consequence, but a cause of insulin resistance and related conditions. They indicate that insulin must cross the endothelium and enter the intercellular space in order to bind to its receptors. In the case of a primary endothelial cell defect, the transendothelial transport of insulin is disrupted and, as a consequence, insulin resistance may develop. In this case, the reduced tissue sensitivity to insulin, as a mechanism of excessive weight, is secondary to endothelial dysfunction. The authors emphasize that the study of markers of endothelial dysfunction has prognostic value [10].

The potential role of NO in the regulation of lipid and carbohydrate metabolism has been demonstrated in several studies, confirming the expression and activity of eNOS in adipose tissue [4, 11]. Pawlik A at al observed a higher BMI in patients with the NOS3 rs1799983 TT genotype in females [12]. We've found only one research performed by a group of co-authors led by Pardo F. [13], which have demonstrated a direct relationship between excessive gestational weight gain (GWG) in women with normal pre-pregnancy BMI and decreased umbilical vein eNOS activity, which is the evidence of endothelial dysfunction. To date, no studies have inspected the association between eNOS Glu298Asp (G894T) and pathological weight gain in pregnant women. The problem of pathological GWG is very actual in terms of prognosis of obstetric and perinatal complications: high risk of hypertensive disorders, impaired glucose tolerance, operative delivery, weight retention and obesity after childbirth [14]. This prompted us to investigate the role of the associations between eNOS Glu298Asp (G894T) endothelial dysfunction gene polymorphisms and metabolic disorders in pathological gestational weight gain pregnancies to prove the mechanism of realization of genetic predisposition in excessive GWG.

THE AIM

The aim of the study was to determine the association between the Glu298Asp (G894T) polymorphisms of the eNOS gene with metabolic disorders in excessive GWG pregnancy.

MATERIALS AND METHODS

The study included 97 pregnant women, who visited the antenatal clinics of Ivano-Frankivsk City Clinical Perinatal Centre (Ukraine) from 2016 until 2019. Inclusion criteria: age 18 years and older, singleton gestation, normal pre-pregnancy BMI (18.5–24.9 kg/m^2), written consent from the patient. Exclusion criteria: age under 18 years old, multiple pregnancy, term of labor up to 37 week, severe extragenital diseases. All women have signed "Informed consent to participate in the study". The research design was approved by the Ethics Committee of the Ivano-Frankivsk National Medical University (№ 93/16 from 01.12.2016).

Pre-pregnancy weight of the patients was 55.8±5.8 kg (95%Cl 54.7–56.9), the average height was 164.7±5.5 cm (95%Cl 163.6–165.8), BMI 20.6±1.6 kg/m^2 (95%Cl 20.3–20.9). The recommended GWG was diagnosed in 33 (34.0±4.8 %), inadequate in 19 (19.6±4.0 %), and excessive in 45 (46.4±5.1 %) patients due to recommendations of the Institute of medicine in the USA (2009) [15] and the Order of the ministry of health of Ukraine № 417 (2011) [16]. The average age of patients was 26.8 ± 2.6 years (95%Cl 26.3–27.3), and did not differ significantly in the groups of women with different GWG (p>0.05). 59 (60.8±5.0 %) of the examined patients were nulliparous, and 38 (39.2±5.0 %) were multiparous women.

Pre-pregnancy weight status of patients was assessed with BMI (kilogram/meter^2) calculated from pre-pregnancy weight and height. Information on the body weight of women before pregnancy was obtained by interviewing patients and medical records. Anthropometry was performed at the first prenatal visit (9.8±1.4 weeks (95% Cl 9.6–10.1)), and before the delivery. Patients were weighted on the electronic scale to the nearest 0.1 kg. Height was measured using a digital stadiometer with an accuracy of 1.0 cm. GWG was evaluated by the difference between the weight before delivery, and pre-pregnancy. The results were compared to the recommended GWG for women with normal BMI – 11.5–16.0 kg – according to the national recommendations [16].

Study of the Gln233Arg polymorphism of the eNOS gene was performed at the State institution "Reference

Table I. The frequency of genotypes of Glu298Asp (G894T) variant of eNOS gene in pregnant women with different gestational weight gain, (x ± SD, n = 97)

GWG	Genotype of eNOS gene		
	GG, n (%)	GT, n (%)	TT, n (%)
Recommended	19 (42.2 ± 7.4)	11 (33.3 ± 8.2)	3 (15.8 ± 8.4) $p_1 < 0.001$
Inadequate	10 (22.2 ± 6.2)	7 (21.2 ± 7.1)	2 (10.5 ± 7.0) $p_1 < 0.05$
Excessive	16 (35.6 ± 7.1)	15 (45.5 ± 8.7)	14 (73.7 ± 10.1) $p_1 > 0.05$, $p_2 < 0.05$

p_1: compared to women-carriers of GG genotype, p_2: compared to women with recommended GWG; GWG - gestational weight gain

Table II. Serum blood levels of lipid metabolism parameters in pregnant women depending on Glu298Asp (G894T) polymorphism of the eNOS gene (x ± SD, n = 97)

Indications	GG, n = 45		GT, n = 33		TT, n = 19	
	I trimester	III trimester	I trimester	III trimester	I trimester	III trimester
TG, mmol/L	1.0 ± 0.3	2.0 ± 0.3*	1.2 ± 0.3	2.4 ± 0.4*	1.3 ± 0.2	3.1 ± 0.4* #
TC, mmol/L	4.9 ± 0.2	5.9 ± 0.3*	4.8 ± 0.2	6.2 ± 0.5*	4.9 ± 0.2	6.7 ± 0.3* #
HDL, mmol/L	1.6 ± 0.1	1.9 ± 0.3	1.6 ± 0.2	1.7 ± 0.3	1.6 ± 0.1	1.6 ± 0.1
LDL, mmol/L	2.4 ± 0.2	3.2 ± 0.2*	2.4 ± 0.2	3.4 ± 0.2*	2.5 ± 0.2	3.8 ± 0.2* #
VLDL, mmol/L	0.3 ± 0.1	0.8 ± 0.3	0.3 ± 0.2	1.0 ± 0.3*	0.3 ± 0.1	1.3 ± 0.3* #

*: compared to the 1st trimester ($p < 0.05$); #: compared to GG – genotype carriers ($p < 0.05$); TG – triglycerides; TC – total cholesterol; HDL – high lipoprotein; LDL - low density lipoprotein; VLDL – very low density lipoprotein

Table III. Carbohydrate metabolism parameters in pregnant women depending on Glu298Asp (G894T) polymorphism of the eNOS gene (x ± SD, n = 97)

Indications	GG, n = 45		GT, n = 33		TT, n = 19	
	I trimester	III trimester	I trimester	III trimester	I trimester	III trimester
Glucose, mol/L	4.3 ± 0.2	4.0 ± 0.5	4.4 ± 0.3	4.3 ± 0.5	4.5 ± 0.3	4.9 ± 0.3
Insulin, μU/ml	8.2 ± 0.9	9.1 ± 1.2	9.3 ± 0.7	11.7 ± 1.6	9.3 ± 0.9	12.8 ± 1.3* #
HOMA – IR	1.5 ± 0.2	1.6 ± 0.5	1.8 ± 0.2	2.2 ± 0.6	1.8 ± 0.2	2.9 ± 0.4* #

*: compared to the lst trimester ($p < 0.05$); #: compared to women GG – genotype carriers ($p < 0.05$)

center for molecular diagnostics of the ministry of health of Ukraine" (Kyiv). Genomic DNA for molecular-genetic studies was isolated from peripheral blood using a commercial "innuPREP Blood DNA Mini Kit" test system (Analytik Jena, Germany) using centrifuge filters. For the determination of the polymorphic variants of the eNOS (G894T, rs1799983) modified protocols were used using the polymerase chain reaction method and the subsequent analysis of polymorphism (Mahmoudi & Alavicheh, 2015). Visualization was done by using the Vitran computer program (Fig. 1). The genotype GG was recorded in the presence of DNA fragments with a length of 248 p.n., genotype GT–fragments with a length of 248, 158, and 90 p.n., and genotype TT–fragments length 158, and 90 p.n.

Lipid profile was established on the basis of the laboratory determination of the concentration of triglycerides (TG) (Triglycerides SpL (SpinLab LLC, Ukraine)), total cholesterol (TC) (Cholesterol SPL (LLC SpinLab, Ukraine)), high lipoprotein, low and very low density (HDL, LDL, VLDL) ("HDL-Cholesterol SPL" (LLC "SpinLab", Ukraine)). To study the carbohydrate status, the level of blood glucose in blood plasma was determined by standard methods, blood insulin content in blood serum by the immune enzyme method (Insulin Test System–2425–300 (Monobind Inc., USA)) with the following calculation of the index of immunoreactivity of HOMA–IR by the formula: glucose (mmol/l) x insulin (μM/ml) / 22.5. The examination was conducted in the first and third trimesters of pregnancy.

The results were statistically analyzed using Statistica 6.0 program pack (StatSoft Inc., USA) and Microsoft Excel statistical analysis package. The parameters are presented as mean arithmetic value, mean standard deviation (x ± SD). For comparison of genotype and allele frequencies between the groups (recommended, inadequate and excessive weight gain) odds ratio (OR), 95% confidence interval (CI), and p – value were obtained. The differences between the selections were considered statistically reliable at $p<0.05$ (Tukey's test).

RESULTS

The analysis of Glu298Asp (G894T) polymorphism of the eNOS gene showed that 45 (46.4±5.1 %) patients were with

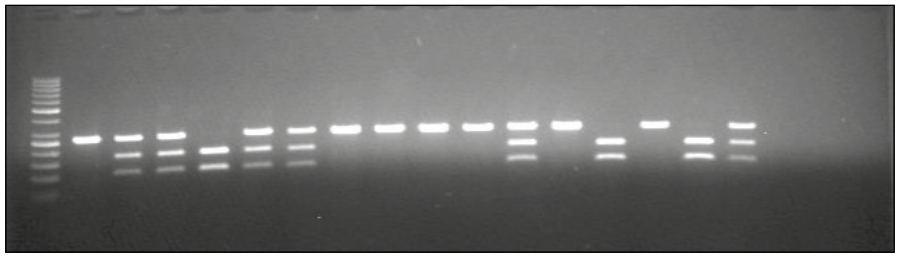

Fig. 1. Electrophoregram of the distribution of DNA fragments of the gene eNOS (G894T).

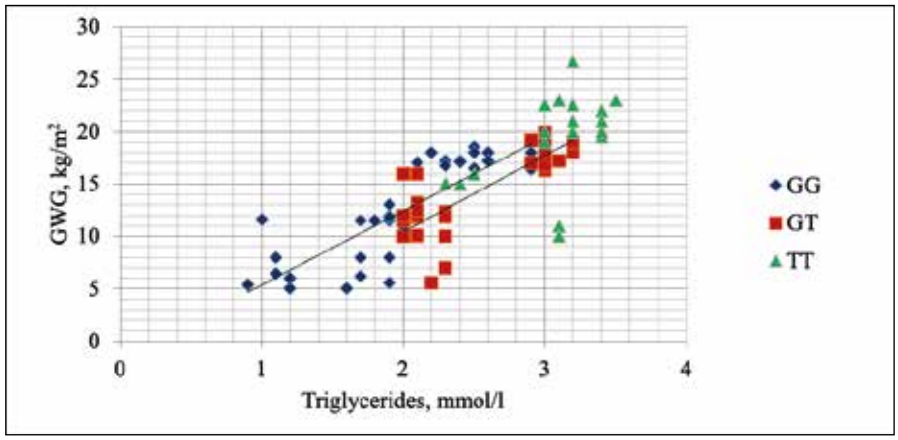

Fig. 2. Concentration of triglycerides in blood of pregnant depending on eNOS gene Glu298Asp (G894T) polymorphisms and GWG in the third trimester, mmol/l

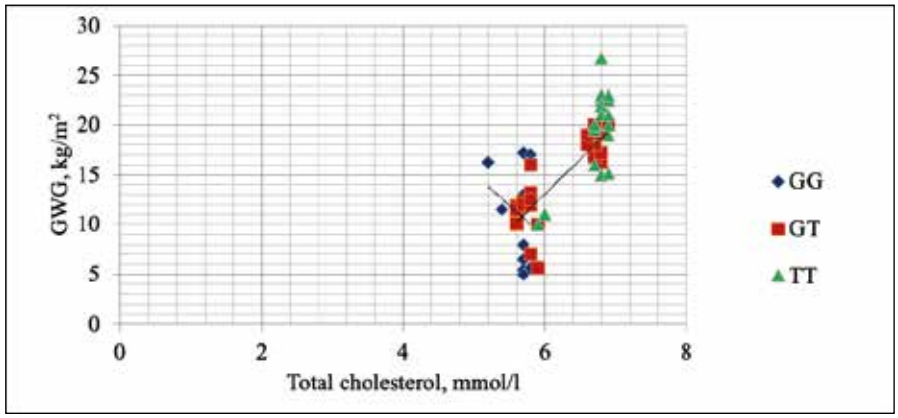

Fig. 3. Concentration of total cholesterol in blood of pregnant depending on eNOS gene Glu298Asp (G894T) polymorphism and GWG in the third trimester, mmol/l

GG genotype, 33 (34.0±4.8 %) – GT genotype, and 19 (19.6±4.0 %) – TT genotype. The frequency of the T alleles in our study was within the limits of the typical Ukrainian population [17].

Pre-pregnancy weight among GG pregnant women was 52.5±4.8 kg, BMI 19.6±0.6 kg/m^2, carriers of GT alleles 55.0±.5 kg, BMI 20.3±1.3 kg/m^2, and TT – genotype women 60.3±5.0 kg, BMI 22.3±1.7 kg/m^2, which were not significantly different (p>0.05). We diagnosed that GWG in late pregnancy in homozygous G–allele carriers was 12.5±2.5 kg (95%CI 11.3-13.4), in women with GT – genotype 14.4±3.1 kg (95%CI 13.1-15.7), and in the homozygous carriers T–allele 19.3±2.3 kg (95%CI 17.5-21.1).

Thus, we diagnosed that GWG in the group of patients with gene eNOS TT polymorphism was significantly higher (1.5-fold) compared to pregnant with GG – genotype (p<0.05). In the group of pregnant women with GT – genotype, GWG was also higher in comparison to GG – genotype, but the difference was not statistically significant (p>0.05). There was no significant difference in weight gain in heterozygous carriers and homozygous T–allele carriers (p>0.05) (Table I).

There was no significant difference in the distribution of women with different levels of GWG in the groups of pregnant GG and GT – genotypes carriers (p>0.05). While in the group of homozygous T–allele Glu298Asp (G894T) eNOS gene carriers only in 3 (15.8±8.4 %) women the recommended and in 2 (10.5±7.0 %) – the insufficient weight gain were found, which were 2.7-fold and 2.1-fold significantly lower compared to carriers of GG – genotype (in both cases p<0,05). Also in the pathological TT – genotype group, excessive GWG was found in 14 (73.7±10.1 %) patients, which was 4.6-fold higher compared to the recommended GWG women' group (p<0.05). Consequently, it is shown that a high degree of risk of excessive GWG was found in pregnant women with TT polymorphism Glu298Asp (G894T) of the eNOS gene (OR=4.52; 95%CI: 1.18-17.32; p<0.05) (frequency distribution of 73.7 %), and the carrier of GT – genotype Glu298Asp (G894T) of the eNOS gene does not affect the level of GWG.

Due to studying the dynamics of laboratory parameters of lipid metabolism in pregnant women, depending on eNOS gene Glu298Asp (G894T) polymorphism, we es-

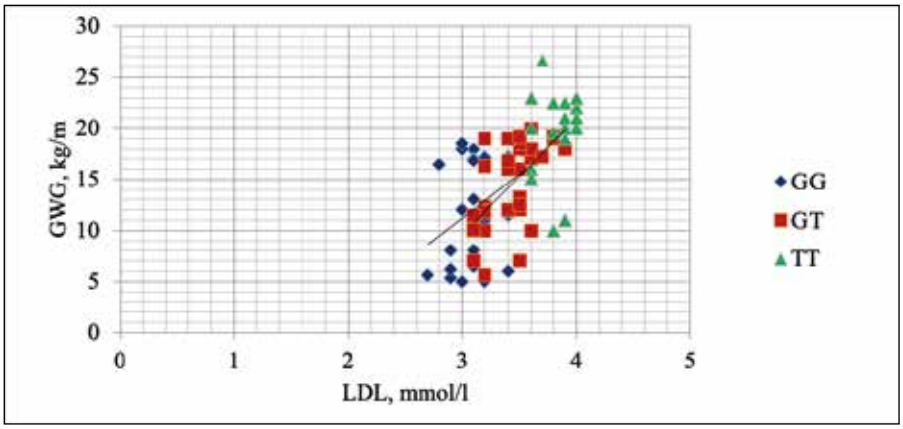

Fig. 4. Concentration of low density lipoproteins in blood of pregnant depending on eNOS gene Glu298Asp (G894T) polymorphisms and GWG in the third trimester, mmol/l

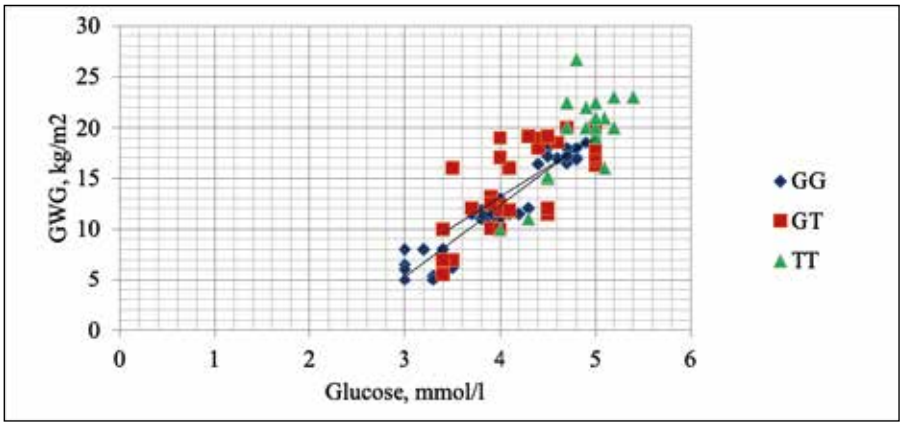

Fig. 5. Concentration of glucose in blood of pregnant depending on eNOS gene Glu298Asp (G894T) polymorphism and GWG in the third trimester, mmol/l

tablished the correspondence of lipid concentration at the beginning of pregnancy with the reference value and the absence of a significant difference in patients with GG, GT, and TT – genotypes ($p > 0.05$) (Table. II).

In late pregnancy, the levels of atherogenic lipids (TG, TC, LDL) were significantly increased in all patients compared to the early terms ($p<0.05$). Thus, in the third trimester TG levels were significantly increased 1.5-fold, TC 1.2-fold, LDL 1.2-fold, VLDL 1.6-fold (in all cases $p<0.05$) in pregnant women with TT polymorphism of the eNOS gene, compared to homozygous G – allele carriers.

We've observed that pregnant women with excessive GWG inherited the TT allele of the eNOS gene associated with higher levels of hypertriglyceridemia, hypercholesterolemia and higher concentrations of LDL compared to carriers of GG and GT alleles (in all cases $p<0.05$) (Fig. 3-5).

Results of the eNOS gene Glu298Asp (G894T) polymorphism study revealed no significant differences in serum glucose level, insulin level and HOMA–IR in women with GG and GT – genotypes (both of them $p>0.05$) (Table III).

However, at the end of pregnancy, the 1.4-fold concentrations of insulin, and HOMA–IR 1.9-fold were significantly higher in the homozygous T – carriers as compared to the GG – genotype pregnant (in all cases $p<0.01$). The genotype TT was associated with a statistically higher insulin level 1.4-fold, and HOMA–IR 1.6-fold before delivery compared to the first trimester ($p < 0.05$), which was not noted in other groups.

The inheritance of the TT allele of the Glu298Asp (G894T) eNOS gene polymorphisms is associated with higher levels of glycemia, hyperinsulinemia, and higher levels of HOMA–IR compared to carriers of GG alleles (in all cases $p<0.05$) in pregnant with excessive GWG (Fig. 6-8).

DISCUSSION

Modern researches have proved that endothelial dysfunction, which is present in overweight, is a result of such interrelated pathogenetic mechanisms as systemic inflammatory reaction, increased oxidative modification of proteins, hyperleptinemia, and is associated with the development of cardiovascular disease, atherosclerosis [2, 3, 12]. However, there is another opinion, that metabolic and hemodynamic changes, and as a consequence of excess body weight, occur and deepen on the background of primary endothelial disease [10].

In this research, we've found an association of polymorphic variants of the eNOS gene Glu298Asp (G894T), which significantly reduce the promoter activity of the eNOS gene, with metabolic disfunction in pregnant, which is most pronounced in the group with excessive GWG. In these individuals, the presence of the TT – allele is accompanied by higher serum levels of TG, TC, LDL compared with carriers of GG – alleles. They are also characterized by a more pronounced violation of tissue sensitivity to insulin, accompanied by hyperinsulinemia and hyperglycemia compared with carriers of GG alleles.

Our conclusions coincide with other scientific reports [18, 19, 21]. Thus, in a recent systematic review and me-

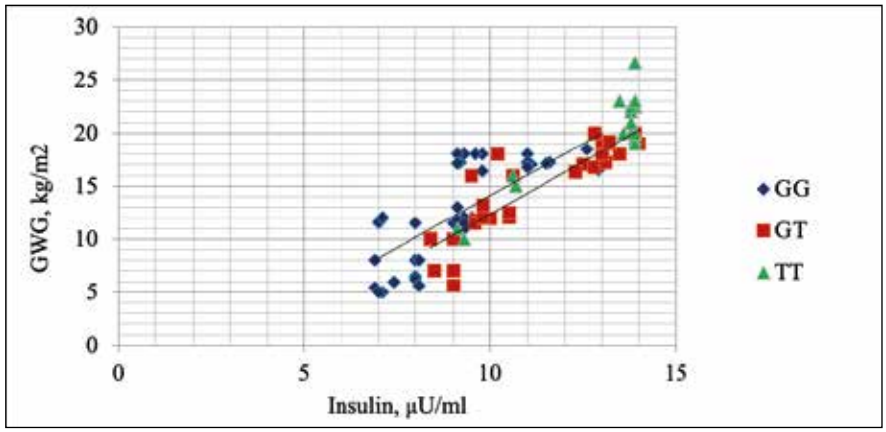

Fig. 6. Concentration of insulin in blood of pregnant depending on eNOS gene Glu298Asp (G894T) polymorphism and GWG in the third trimester, μU/ml

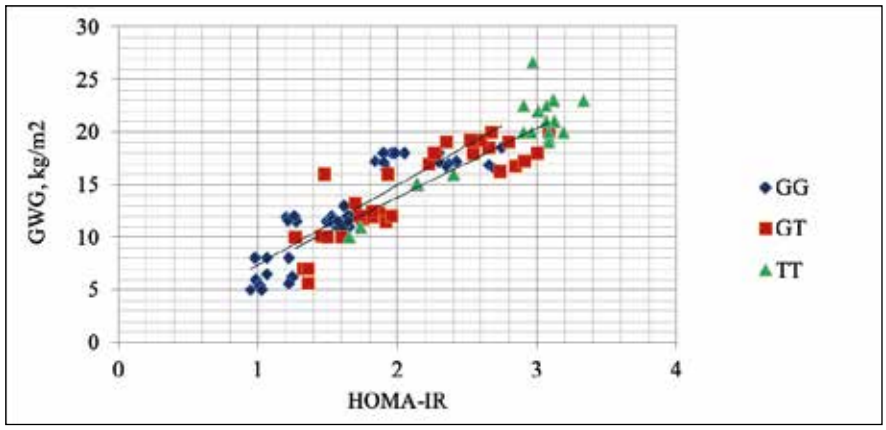

Fig. 7. Insulin resistance index of pregnant depending on eNOS gene Glu298Asp (G894T) polymorphism and GWG in the third trimester

ta-analysis of 59 studies by Luo Z., et al. [18] demonstrated significantly higher levels of TG, TC, LDL, as well as lower levels of HDL in the T – allele carrier compared to individuals with GG – genotype of the eNOS gene. According to the authors, the rs1799983 polymorphism modulates the plasma lipids levels in through the mediation of NO. Decreased NO levels in the blood cause an increase in blood pressure, endothelial dysfunction and oxidative stress, which is likely provoke the development of dyslipidemia.

Teixeira TG., et al demonstrated the associations between G894T SNP and plasma triglycerides in Caucasian patients with obesity [19]. They shoved that eNOS Glu298Asp gene polymorphism influences body composition and biochemical parameters but not the nitric oxide response to eccentric resistance exercise in elderly obese women. However, Fattakhov N., et al revealed no associations for G894T with biochemical parameters of lipid and carbohydrate metabolism in metabolic syndrom patients [20]. These inconsistencies could perhaps be due to differences in sample size, age, study design, and interethnic differences within the distribution of NOS3 variants.

Sansbury B., at al. also demonstrated higher BMI, waist circumference, skin folds, and the percentage of fat mass in women, carrying the TT – allele Glu298Asp (G894T) of the eNOS gene polymorphism, which is associated with decreased adipose tissue lipolysis. Endothelial NO synthase is likely to enhance fat oxidation and lipid synthesis in tissues such as liver, skeletal muscle, and fat. Relatively low levels of fatty acids and triglycerides in blood plasma found in models with excessive level of eNOS, which corresponds to this mechanism [21].

There are scientific suggestions, that eNOS has a potent anti-obesity effect by improving tissue sensitivity to insulin. Becerril S., et al. [22] reported that the beneficial effect of eNOS on glucose metabolism and insulin sensitivity is due to its ability to stimulate the transport of insulin and glucose to key peripheral tissues, and to regulate gluconeogenesis [23].

Imbalance in the synthesis of lipoproteins in women with excessive GWG, manifested by hypertriglyceridemia with a predominance of atherogenic fractions (LDL, VLDL) stimulates excessive accumulation of lipids in the endothelium. As a result, damage to endothelial cell membranes is deepened and the synthesis of enzymes, required for the secretion of vasodilators, is inhibited [1]. Adank M., et al. proved that endothelial cells loaded with lipid complexes become more vulnerable to free radical damage. In addition, the low serum HDL level of such patients is able to stimulate a decrease in prostacyclin levels. These changes can lead to systemic endotoxicosis, generalized endothelial dysfunction with changes in vascular lumen in the direction of vasoconstriction, and subsequently – to thrombosis and tissue ischemia, which is clinically manifested in the gestational complications [24].

Researches about eNOS Glu298Asp polymorphisms as the risk factor of the pathological GWG are limited. Thus, our results indicate that the eNOS Glu298Asp polymorphisms associated with endothelial dysfunction is the

pathogenetic basis for the development of overweight, which deepens the knowledge of understanding the genetic basis of pathological GWG. On the other side, excessive GWG in women with normal pre-pregnancy BMI can be considered as an early manifestation of a genetic predisposition to endothelial dysfunction.

CONCLUSIONS

Decreased activity of the eNOS gene in the presence of pathological alleles is the initiator of impaired lipid and carbohydrate metabolisms and, as a consequence, excessive GWG. A high risk of excessive GWG was found in pregnant women with TT polymorphism Glu298Asp (G894T) (OR=4.52; 95%CI: 1.18-17.32; $p<0.05$) (frequency distribution of 73.7 %). Endothelial dysfunction is a pathogenetic link of excessive body weigh in pregnancy.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

PROGNOSTIC CRITERIA FOR THE SELECTION OF INDIVIDUALS WITH DIFFERENT HEAT SENSITIVITY

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ABSTRACT

The aim: To establish criteria for individual thermal sensitivity in almost healthy individuals aged 17-20 years.

Materials and methods: We surveyed 150 people aged 17-20 years. Heat sensitivity was previously assessed using our adapted questionnaire «Levels of heat sensitivity», in which according to the scale the number of points 0-6 indicates reduced heat sensitivity, 7-16 points - increased). All respondents underwent thermal testing. The subjects had auscultatory determination of pulse rate and blood pressure, after which the hands of both hands were immersed in a container with warm water (45° C) for 3 minutes. At the 2nd minute, pulse rate and blood pressure were determined. this procedure was repeated after the hands were removed from the water, and every 2 minutes until the pulse and blood pressure recovered over time. Before and after the heat test, a mathematical analysis of heart rate was performed using a computer software package, according to the manufacturer›s instructions.

Results: After conducting a thermal test in people with hypersensitivity, an increase in the average values of heart rate and blood pressure. In the group of people with lower sensitivity to heat, heart rate and blood pressure after the heat test decreased or did not change. As a result of statistical processing of data from mathematical analysis of heart rate, it was found that in persons with hypersensitivity after the thermal test increased activity of the sympathetic nervous system, while in subjects with reduced sensitivity to heat showed a slight advantage of parasympathetic nervous system.

Conclusions: Increased activity of the sympathetic nervous system in response to heat in people with hypersensitivity indicates the development of stress, and given the current trend towards long-term and constant increase in average annual temperature, this will lead to overstrain of regulatory mechanisms, depletion of functional reserves and reduced adaptability . Therefore, people with high heat sensitivity are particularly vulnerable to global climate change.

KEY WORDS: heat sensitivity; individual sensitivity; thermal factor

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INTRODUCTION

Information about the ambient temperature comes to the thermoregulatory centers from thermoreceptors located in different parts of the skin [1-3]. This system is organized by the unity of interaction of peripheral and central departments [4-6]. The center of thermoregulation is located in the hypothalamus, where is the center of the autonomic nervous system [7-9]. This ensures the body's ability to maintain the stability of the internal environment even with significant changes in the external environment [3, 10].

In any population, not all individuals respond equally to environmental factors [6, 11]. Currently, there is a lot of scientific information on the effects of cold stress on the human body [7, 12, 13]. In the literature analyzed by us, we found only isolated data on individual reactions of people to the effects of heat [2, 5].

We have not found a single, clear and easy-to-use algorithm for assessing the body's sensitivity to heat. This issue is quite relevant in today's period, the era of global warming, which is recognized by the Intergovernmental Group of Experts as a catastrophe that is rapidly approaching and causes irreversible damage not only to the environment but also to human health [7].

THE AIM

The aim of the work was to establish criteria for individual thermal sensitivity in almost healthy individuals aged 17-20 years.

MATERIALS AND METHODS

We surveyed 150 people aged 17-20 years. Heat sensitivity was previously assessed using our adapted questionnaire «Levels of heat sensitivity», in which according to the scale the number of points 0-6 indicates reduced heat sensitivity, 7-16 points - increased). To confirm the results obtained during the survey in the future, all subjects underwent a thermal test. Before the study, the temperature of the room in which the study was conducted was measured

using an electronic thermometer «Omron Gentle Temp 720 (MS-720-E)». Before the study, the temperature of the room in which the study was performed was measured using an electronic thermometer «Omron Gentle Temp 720 (MS-720-E)».

In the examined people, the pulse rate and blood pressure were determined by auscultatory method using the MMP-60 device, after which the hands of both hands were immersed in a container with warm water (45 ° C) for 3 minutes. During this, the pulse rate and blood pressure were determined at the 2-nd minute. this procedure was repeated after the hands were removed from the water and every 2 minutes until the pulse and blood pressure were restored with time.

Before and after the heat test, a mathematical analysis of heart rate was performed using the computer software complex «Spectrum +» (LLC «Spectrometer-Ukraine», Kharkiv, Ukraine), according to the manufacturer's instructions. 5-minute segments of rhythmograms were recorded. Statistical and spectral methods were also used, as well as cardiointervalography according to R.M. Baevsky [14].

Analysis and processing of the survey data were performed using the program «Microsoft Excel». To compare the significance of the differences between the two samples, nonparametric statistics were used, in particular, the Mann – Whitney rank test at a critical significance level of $p < 0.01$. Also, statistical data processing was performed using «Statistica 12» (used the method of data mining, CART algorithm, classification trees).

Our research does not contradict the adopted bioethical norms of the Declaration of Helsinki, adopted by the General Assembly of the World Medical Association on Human Rights, as well as the International Code of Medical Ethics and the laws of Ukraine. They can be used in scientific work (decision of the commission of bioethics of Ternopil National Medical University of the Ministry of Health of Ukraine, protocol № 66 from November 1, 2021) [15].

Our research was conducted on the basis of the Ministry of Health of Ukraine certified laboratory of psychophysiological research of the Department of Physiology, Bioethics and Biosafety of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Certificate № 003/18).

RESULTS

Analysis of data that were analyzed and obtained using the questionnaire «Levels of heat sensitivity» showed that 56 people (37.3%) received a total of 7 to 16 points, indicating increased sensitivity, and 94 people (62.7%)) received from 0 to 6 points, indicating reduced heat sensitivity. Of those who were sensitive to heat, 78% preferred room and ambient temperatures below 20 ° C, while 84.2% of those who were less sensitive to heat felt comfortable at room temperature above 20 ° C and ambient temperature of 20-25 ° C.

Among the general symptoms reported in question № 5, surveyed people with hypersensitivity to heat, on hot days, reported a predominance of such manifestations as: weakness, drowsiness, difficulty concentrating, increased sweating, tachycardia, increased thirst, reduced efficiency, compared with persons with reduced sensitivity to heat.

After the survey, all subjects underwent a thermal test. The average value of the pulse rate in the initial state in persons with hypersensitivity was 79.46 ± 0.73 bpm, and blood pressure: systolic -120.53 ± 0.96 mmHg, diastolic - 75.89 ± 0.74 mmHg. In the group with reduced heat sensitivity, the initial value of heart rate was 84.06 ± 0.82 bpm, and blood pressure: systolic - 122.44 ± 0.85 mm Hg, diastolic - 77.97 ± 0.63 mm Hg. A statistically significant difference between the initial values of heart rate in individuals with high and low heat sensitivity ($p<0.05$).

At 2 minutes of staying hands in warm water, the average value of heart rate in persons with hypersensitivity was 84.72 ± 2.02 bpm, blood pressure: systolic 125.92 ± 0.84 mm Hg, diastolic - 76.82 ± 0.86 mmHg. In subjects with reduced heat sensitivity, the mean value of heart rate at 2 minutes was 77.54 ± 1.18 bpm (statistically significant difference compared with the value of heart rate at 2 minutes in persons with hypersensitivity, as well as compared to baseline values pulse rate in the examined both groups, $p<0.05$), blood pressure: systolic - 115.43 ± 0.86 mmHg., diastolic - 75.59 ± 0.62 mmHg.

After the thermal test in persons with hypersensitivity, the pulse rate was 82.92 ± 4.08 bpm, which exceeded the initial value by 4.35% ($p<0.05$), the blood pressure changed as follows: systolic increase by 4.4% (mean value - 125.89 ± 0.92 mm Hg), diastolic increase by 1% (mean value 76.58 ± 0.56 mm Hg). In the group with reduced heat sensitivity after the thermal test, the pulse rate was - 76.87 ± 2.28 bpm, which was lower than the initial data by 8.5% ($p<0.01$), and blood pressure values underwent such changes : systolic decreased by 5% (mean 116.27 ± 0.85 mm Hg), diastolic decreased by 4% (mean 74.78 ± 0.73 mm Hg).

The recovery time of the pulse after the thermal test to baseline was in the group with high heat sensitivity an average of 270 seconds, in persons with low heat sensitivity this figure was 295 seconds.

The recovery time of blood pressure after the test to baseline was in the group with hypersensitivity: systolic - 363 seconds, diastolic - 261 seconds. In the group with reduced heat sensitivity, the indicators returned to baseline slightly faster: systolic - 275 seconds, diastolic - 235 seconds (statistically significant difference in the recovery of systolic blood pressure, $p < 0.05$).

DISCUSSION

Analyzing the above, we can say that the reaction to temperature stimuli is characterized by an individual that is consistent with our results.

As a result of spectral analysis of mathematical analysis of heart rate to heat test, it was found that in the group with reduced heat sensitivity significantly higher TP (Total Power), SDNN (Standart Deviation of all Normal to Normal Intervals), RRNN (The average duration of RR

Table I. The results of the analysis of heart rate variability and cardiointervalography according to the method of R.M. Baevsky in subjects with different heat sensitivity

Examined indicators	The results of the analysis in persons with reduced heat sensitivity, M ± m		The results of the analysis in persons with hypersensitivity, M ± m	
	Before the thermal test	After the thermal test	Before the thermal test	After the thermal test
RRNN, ms	754,18±13,26	793,49±13,97*	618,57±8,45**	615,53±7,85***
SDNN, ms	72,63±2,99	83,21±3,61*	51,86±2,32**	47,57±1,85*,***
TP, s2/Hz	6678,79±405,46	7134,58±614,45	4322,18±314,45**	4233,68±320,47***
HF%	33,64±1,75	36,04±1,65*	27,36±1,66**	23,36±1,16*,***
LF%	36±0,97	31,90±0,87*	31,36±1,37**	37,89±1,36*,***
VLF%	30,30±1,31	30,78±1,01	33,21±1,11	34,46±1,27
LF/HF	1,93±0,30	1,83±0,18	1,49±0,17**	1,80±0,18*
Mo, s	0,75±0,02	0,80±0,02*	0,61±0,01**	0,68±0,01*,***
AMo, %	41,08±0,92	40,69±1,02*	38,16±1,26**	42,77±1,32*,***
ΔX, s	0,49±0,04	0,52±0,02*	0,37±0,02**	0,31±0,01***
SI, n.u.	73,83±8,78	58,50±8,22*	82,12±4,43**	139,96±12,11*,***

Note: * - statistically significant difference between before and after the heat test in the groups with reduced and increased heat sensitivity (p<0.01), **- statistically significant difference in indicators before the thermal test in persons with high compared to low heat sensitivity (p <0.01), ***- statistically significant difference of indicators after thermal testing in persons with increased compared to reduced heat sensitivity (p <0.01)

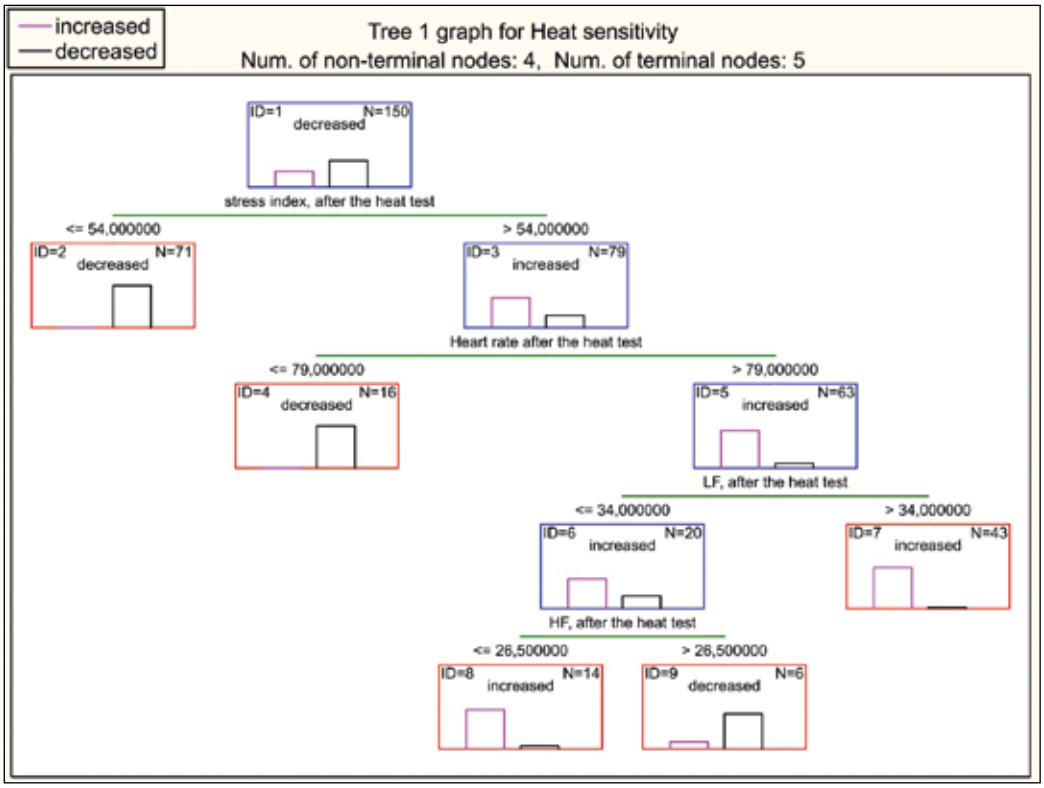
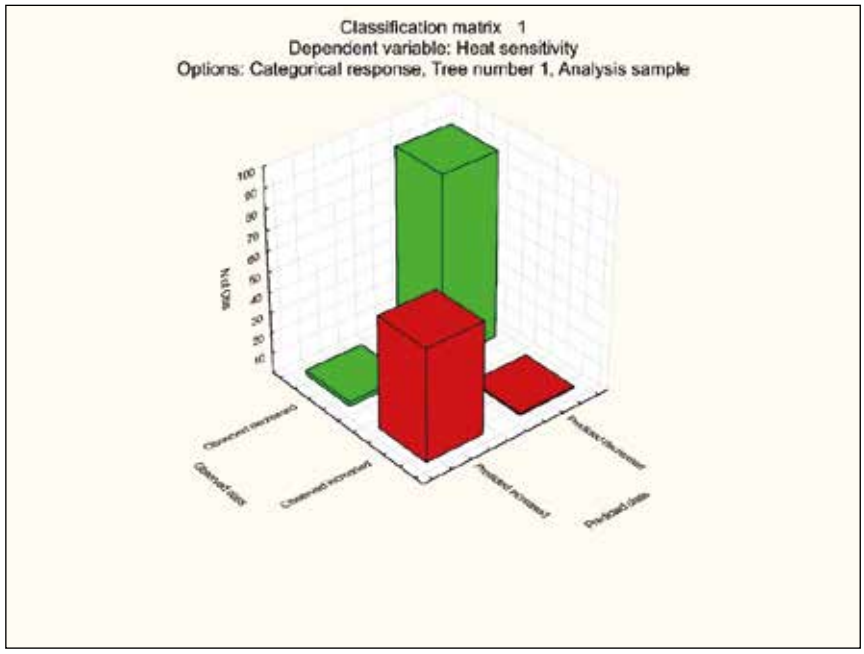


Fig. 1. Decision tree based on the data of the thermal test, spectral analysis of heart rate and cardiointervalography according to R.M. Baevsky in order to identify increased and decreased heat sensitivity.

intervals), HF (high frequency power) , ΔX (Variational scope) and significantly lower SI (Stress index) (p <0.01), compared with the group with increased heat sensitivity, which indicates the predominance of the parasympathetic nervous system. In the group with increased heat sensitivity, significantly higher indicators of SI and significantly lower indicators of TP, SDNN, RRNN, HF, ΔX (p <0.01), which indicates the predominance of the sympathetic nervous system (Table I).

The results of mathematical analysis of heart rate after heat test show that people with low heat sensitivity are dominated by parasympathetic effects, as indicated by an increase in mean values such as RRNN, SDNN, HF, ΔX and a decrease in indicators such as LF (low frequency power), LF/HF (vagosympathetic balance), AMo (mode amplitude), SI. These data indicate a high level of functional reserves of the body, which provides a sufficient degree of adaptation. Subjects with hypersensitivity after the heat test



Figt 2. The results of the distribution of CART algorithms of heat sensitivity levels.

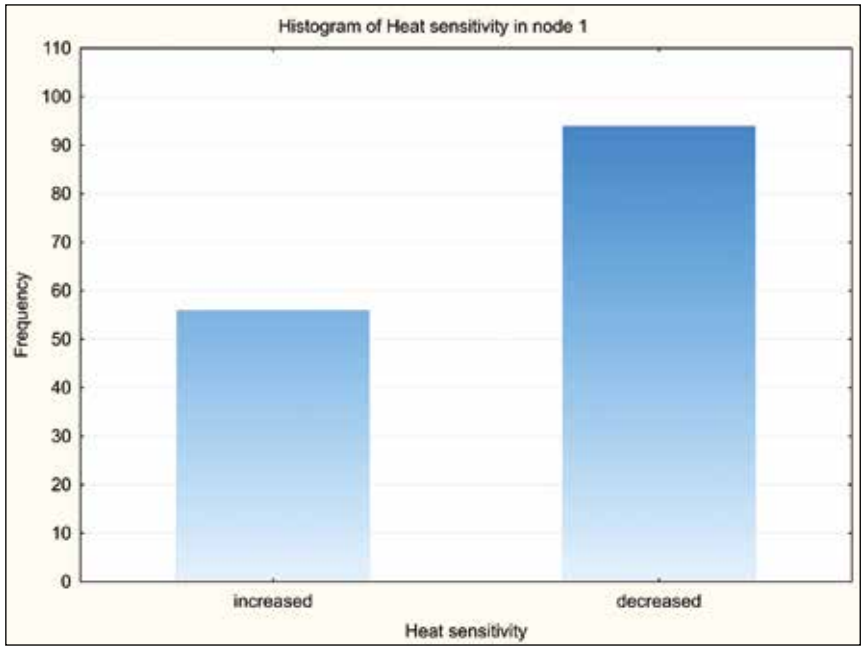


Fig. 3. Histogram by levels of heat sensitivity after statistical analysis (Node 1 of Decision tree).

showed increased activity of the sympathetic autonomic nervous system, as evidenced by an increase in mean values such as LF, VLF (Very low frequency power), LF/HF, AMo, SI and a decrease in RRNN, SDNN, HF, ΔX). The obtained results indicate the stress of regulatory mechanisms, and adaptation occurs through the involvement of functional reserves of the organism (Table I).

To process the obtained data in order to find non-obvious, objective and useful patterns of classification of persons with different heat sensitivity, we used the method of Data Mining analysis and its direction - Decision tree (Decision tree, branching algorithm - CARD) (Statistica 12). This method allows you to better visualize the results of the study and interpret them more clearly. The CARD algorithm for finding the best way to classify data performs a sequential check of all possible combinations of independent variables.

Figure 1 clearly shows that the first parameter that separates increased and decreased heat sensitivity is the stress index after the heat test. If the stress index is less than 54.0 conventional units, the heat sensitivity is reduced in 71 cases with a probability of 100 %. When the stress index is more than 54.0 conventional units, in 52 cases with a probability of 65.8 % heat sensitivity is increased, and in 27 cases with a probability of 34.2 % heat sensitivity is reduced.

At a heart rate, after the thermal test (Heart rate after the heat test), less than 79 beats per minute, in 16 cases with a probability of 100 % reduced sensitivity. If the value of this indicator is more than 79 bpm, then with a probability of

88.9 % in 56 subjects increased heat sensitivity, and with a probability of 11.1 % in 7 cases is reduced.

The next separation criterion is LF, %, after the heat test (LF, after the heat test). When its value is more than 34 %, with a probability of 97.7 % in 42 subjects increased heat sensitivity, and in 1 case decreased with a probability of 2.3 %. At that time if LF, % after the heat test is less than 34 %, then with a probability of 30 % in 6 cases the heat sensitivity is reduced, and with a probability of 70 % in 14 - increased.

Analyzing the last branch of the decision tree, according to HF, %, after the heat test (HF, after the heat test), we noted that when its value is more than 26.5 %, then with a probability of 83.3 % in 5 cases the heat sensitivity is reduced, and in 1 - increased (probability 16.7 %). When the value of this indicator is less than 26.5 %, with a probability of 92.8 % in 13 cases the heat sensitivity is increased, and in 1 case it is reduced (probability 7.2 %).

In Fig. 2 graphically depicts the results obtained after analyzing the decision tree (see Fig.1). According to the results of the distribution, 57 cases out of 150, ie 38.0 % of the surveyed, belong to the group with high heat sensitivity, the error is 1.33 % (2 cases). The group with reduced heat sensitivity included 93 cases (62.0 %), error 0.67 % (1 case).

In Fig. 3 shows the statistical analysis of the data of the questionnaire «Levels of heat sensitivity», heat test, spectral analysis of heart rate and cardiointervalography according to the method of R.M. Baevsky. It was found that of the 150 subjects aged 17-20 years, 56 people had increased heat sensitivity (37.3 %), and 94 decreased (62.7 %).

CONCLUSIONS

1. Based on the analysis of the data of the questionnaire «Levels of heat sensitivity» it was found that out of 150 subjects aged 17-20 years - 56 people with high heat sensitivity (37.3 %) and 94 with low (62.7 %), which indicates individual heat sensitivity .
2. As a result of data processing of mathematical analysis of heart rate before the heat test, the predominance of the parasympathetic nervous system was predominant in the group with reduced heat sensitivity, and the predominance of the sympathetic nervous system in the group with hypersensitivity to heat.
3. After conducting a thermal test in persons with hypersensitivity, an increase in the average values of heart rate and blood pressure. In the group of people with reduced sensitivity to heat, heart rate and blood pressure after the heat test decreased or did not change.
4. As a result of statistical processing of data from mathematical analysis of heart rate after heat test, it was found that in persons with hypersensitivity increased activity of the sympathetic nervous system, while in subjects with reduced sensitivity to heat found a predominance of parasympathetic nervous system.
5. The method of analysis Data Mining (Decision tree algorithm) showed that important parameters for the division of individuals into high and low heat sensitivity are the Stress Index after the heat test, heart rate after the heat test, LF% and HF% after heat exposure.
6. Increased activity of the sympathetic nervous system in response to heat in people with hypersensitivity indicates the development of stress, and, given the current trend towards long-term and constant increase in average annual temperature, this will lead to overstrain of regulatory mechanisms, depletion of functional reserves and reduction of adaptive capabilities. Therefore, people with high heat sensitivity are particularly vulnerable to global climate change.
7. The established criteria of individual thermal sensitivity can become a theoretical basis for the formation of risk groups of persons who are potentially more vulnerable to global warming.

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ORIGINAL ARTICLE

MANAGERIAL COMPETENCE DEVELOPMENT IN THE CONTEXT OF PHILOSOPHY OF EDUCATION

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ABSTRACT

The aim: To analyze the problem of university management in the cultural and philosophical aspects.

Materials and methods: The research was conducted in the form of a written questionnaire. Students were supposed to give a single answer to each question. The survey was conducted in September 2017-2018 and 2018-2019 academic year at Ternopil National Medical University. It encompassed 274 second-year Ukrainian students of medical, dental and pharmaceutical faculties and Institute of Nursing and 228 first, second and third year foreign students, who have come from the USA and study at the faculty of foreign students and International School of Medicine. Although the number of foreign students who participated in the survey was small, we believe that the number of respondents was sufficient enough to draw conclusions about the general trends in the issue under study.

Results: Analysis of responses given by Ukrainian and American students allowed us to single out certain trends and specific differences in their outlook and professionally relevant persuasions and attitudes. A positive peculiarity is that the main motive that prompted Ukrainian students to choose a medical profession was that they believe it to be humane and noble (36%). Despite this, many students consider other important criteria when choosing a profession; particularly that it is prestigious and well-paid (21 % and 13 % respectively). The negative sign is that relatively big amount of students in the comparison with Western countries (the USA in particular), indicate that the occupational choice was influenced by their parents' desire (14 %).

Conclusions: The role of pedoetology as one of the priorities of pedagogical science and practice, which should generalize managerial aspects, has been proved. The experience of university education of the USA in the questions of educational management has been analyzed. An overview of the basic concepts relevant to the management of American higher education has been conducted. The features of university management in the USA in modern conditions have been characterized. The differences in the field of professionally significant value orientations between Ukrainian and American students have been investigated. The emphasis is given to the role of teachers' philosophical culture in successful teaching and learning.

KEY WORDS: culture, management, university, student

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INTRODUCTION

Problems related to higher education reformation in accordance with high standards of quality and management become crucial in modern theory and practice. At the same time, the process of developing higher education management is slow and accompanied by many difficulties, problems and contradictions. Therefore, the objective need to address the pressing problems in the field of higher education management in European countries consists in the study and transformation of the experience of leading countries in this area. Significant interest is also caused by the experience of countries that in the new historical conditions try to comply with international standards, combining domestic experience with the achievements of their foreign colleagues. A relatively new aspect of research is the combination of scientific research by specialists in various scientific disciplines. In this process, a special integrative role is played by philosophy. It is no coincidence that the authors of one of the best guidebooks on management philosophy in Ukraine made a general conclusion that "the successful solution of extremely difficult tasks related to assurance of the proper management efficiency, its compliance with the nature of social changes and the needs of social systems themselves in quality management is impossible without deep philosophical analysis as the conditions of functioning and development of society, and the very phenomenon of management. Therefore, the interest in the management philosophy as the theoretical, ideological, methodological and predictive basis of management activity is not accidental ..." [1].

Summarizing the results of the research, this phenomenon can be explained by a number of reasons. Firstly, the crisis in humanitarian disciplines, which do not always keep up with dynamic social, economic changes, new challenges, and lack of predictability of the youth environment, is obvious. We consider the crisis as a prerequisite for further development.

Secondly, there are significant difficulties in defining the system of basic concepts. After all, in each country, the essence of such basic concepts as a person, individual, person, education, education, pedagogy, etc is defined differently. Thirdly, scientific knowledge achieves the level of science only when scientists approach the level of the regularities of the process. We use the notion of "regularity", as it is rather difficult to talk about the laws of human development and community. Regularities as open, relatively stable relationships between phenomena allow us to describe, explain reasons, and predict the development of events [2]. Obviously, without a reasonable predictive assessment, it is difficult to achieve the desired result. Fourthly, researchers state the presence of the difficulties at the methodological level. One of the examples is comparative pedagogy. Without substantiation of the main criteria for comparison and principles of implementation, rational introduction of achievements of other countries, a combination of historical experience with innovative achievements is not possible. All these questions require a philosophical generalization.

PROBLEM OF RESEARCH

The analysis of anthroposociogenesis enabled the researchers to make important generalizations: "1. A man as an integrity, open to the world, is characterized by the following components: natural (biological) level; spiritual level; social (public) level; generic (universal) level and level of space. In unity, they provide the essence of a man, special and unique, fundamentally different from the essence of other manifestations of life on the Earth. 2. The ontological basis of human existence is its essential power, the ability to carry out meaningful activity. 3. The main quality of a man is their human content (shame, conscience, compassion, solidarity, reverence for nature, perception of life as a supreme value). 4. A man belongs simultaneously to two worlds – phenomenal and intelligible (Latin intelligentsis – conceivable, cognitive). A man constantly overcomes their phenomenal character, creating their own face and content on the path of dialogue and overcoming contradictions on the levels of I and I; I and YOU; I and WE; I and THEY; I and NATURE; I and SOCIETY; I and WORLD. 5. In search of oneself outside of the nature, a man uses game and creativity. They design the desired image and take the risk in making a choice, leaving behind all other possible options. 6. A man lives in a certain cultural paradigm, which asks for a certain orientation and provokes certain associations. 7. A man lives in a state of incompleteness. They have the past, the present and the future. Being a subject of the possible, a man constantly demonstrates the state of dissatisfaction through their existence, being aware of the special nature of their life as one of the levels of self-organization of the the world" [3].

Studying foreign experience allows us to conclude that one of the priorities of pedagogical science and practice is the professional activity of teachers and university instructors. Scientists even single out a separate unit of pedagogy with eloquent name "pedoeology". In our opinion, this science, at the theoretical level, should generalize the managerial aspects, obviously taking into account age and gender characteristics.

As an example, let us look at the work of Professor Jolanta Szempruch, who, by the way, is well known not only in Poland, but also in Europe. We will analyze the latest books dedicated to this problem as an example. First of all, let us pay attention to the structure of the books. In the monograph "Pedoetology" [4], this science is considered as a subdiscipline of pedagogy, it analyzes the peculiarities of qualification, competence, personal qualities of the teacher, moral aspects, activities in the conditions of society's transformation. It must be noted that each section is summarized by a list of discussion questions. Naturally, the first chapter is devoted to the historiography of the problem. The term "pedoeology" was first used by the Italian psychologist Pietro Eusebietti, and later, this problem was analyzed by E. Claparede and E. Meumann.

An emphasis is made on the congress of the International League for New Education in Nice which was held in 1932. The discussion unfolded around the problems identified by Professor John B. Watson: methods of selection for the pedagogical profession, a cooperation between teachers' education and schools, individualization of students' training, active methods used by educational institutions for the development of amateur candidates, organization of continuous contact of students with social life of their environment, focusing the teaching on the "centers of life", development of skills of using practical methods of learning within and beyond school, adherence to democratic principles (freedom), organization of methodological and pedagogical practices, development of independent thinking, the desire for comprehensive personality development, care for intellectual, social, artistic, political development, and development of the image of the ideal of the teacher [4].

For theoretical foundations of pedagogy, it is important to distinguish between four main aspects of methodological, involving the use of theoretical constructs, worked out in philosophy, psychology, sociology, as well as modeling; and epistemological approach that involves quantitative and qualitative assessment of phenomena, as well as the use of empathy, introspection, interpretation; axiological assessment of such cognitive values as truth, rationality, objectivity, as well as with such values as beauty, tolerance, goodness, dialogue, cooperation, achievements of praxeology, first of all, efficiency and cost-effectiveness assessment.

Professional knowledge is considered in three main aspects: knowledge of the world, relations "I-world", and self-cognition. It is interesting that each of the three aspects involves three levels: emancipation and practical-moral knowledge; technical and instrumental knowledge, practical knowledge, aimed at empathic understanding of other people and social processes [4].

Logical continuation of the above-mentioned ideas is six aspects of professional competence: personal competence, interpretive and communicative competence; competence of cooperation; pragmatic competence, informational and medial competence, creative and critical competence.

Among the new trends, problems of diagnosis, socialization, personal development, security, openness to new technologies, perceptions, representatives of different cultures, peoples, races, world outlook, life in harmony with nature; healthy lifestyle, information and reform activities, creative activity are identified [4]. Author's interest to the role of authority seems to be promising as well.

It is worth to single out the successful and traditional combination of the actual pedagogical and psychological aspects in Polish pedoetology. In particular, attention is drawn to the shock of a professional start, insecurity, loneliness, internal anxiety, and stress. The author considers five styles of conflict resolution: struggle, subordination, avoidance, compromise, cooperation.

The assessment of the personality as a leader who has developed such qualities as courage, concentration, attention, communicative ability, ability to work in a group, belief in oneself is seen as perspective as well. Among different forms of self-improvement, attention is focused on joint projects, mentoring, coaching, discussions, benchmarking.

Specialists' attention will drawn by the criteria of "person of success" and "person of defeat". Furthermore, it is worthwhile to highlight a chapter devoted to stress issues and professional burnout. The emphasis is given to the problems of emotional burnout, depersonalization, and a reduction in the sense of personal achievement.

The symptoms of professional burnout deserve particular attention. Five groups of symptoms are distinguished: affective, cognitive, somatic, behavioral, and motivational. These five groups, in turn, are divided into three subgroups according to individual, interpersonal, organizational aspect.

For example, cognitive symptoms on an individual level manifest themselves in hopelessness, powerlessness, pocrucie osacrenia, feeling of defeat, low self-esteem, concentration on oneself, feelings of guilt, thoughts about suicide, memory problems. Affective symptoms at the organizational level are manifested in cynicism, loss of respect for employees and leadership.

Particular interest among teachers will be caused by a system of stress situations management. On the basis of seven aspects of stress (relationships between people, emotional reactions, cognitive responses, physical manifestations, reactions due to the use of tasks, stress caused by the organization) there are suggested definite methods of assistance. For example, in order to overcome the stressful situations caused by the implementation of tasks, it is proposed: to define priorities and detailed planning, to transfer part of the responsibilities to other people, to rationally use the time.

There is no doubt about the importance of the problem of training people with special needs which is given much attention by the author.

RESEARCH FOCUS

Of particular interest is the experience of the USA university management. The United States were one of the first in the world to announce their focus on improving the quality and efficiency of higher education. Particular attention is drawn to the achievement in the management of the quality assurance of higher, in particular, medical education, where for decades the idea of orientation of all levels of education to the person and their needs has been developed and implemented.

The quality of doctors training in the United States depends on the inextricable connection, interaction and cooperation between public, professional and social subjects of higher education. The USA is one of the first to begin reforming the education system, and the current state indicates a number of positive achievements. To improve the quality management of higher education in Europe, adaptation of American experience must be considered which is realized at the technological level, creating the opportunity for correction of initially set goals, content, methods of interaction, search and application of flexible and rational decisions.

Due to its complexity, the problem of management of education is multidisciplinary. Its consideration can and must be based on the theoretical, conceptual and methodological basis of pedagogy, theory of management, praxeology, systemology, sociology and other scientific disciplines.

For a successful study of the peculiarities of educational system management, a clear understanding of the basic concepts of American higher education is necessary, that is, the study of the problem outlined above requires an analysis of its conceptual apparatus. The development, systematization and improvement of the conceptual system of pedagogy are among the permanent and constantly relevant problems of science.

In the context of the analysis of the peculiarities of higher education management in the United States, it is appropriate to define the essence of the concept of management. A considerable number of works are devoted to problems of management in the field of education, as well as analysis of various organizational and managerial aspects of educational activity in domestic and foreign scientific literature. However, the vast majority of works on this topic, despite the fact that their authors formulate the topic as an analysis of management processes in the research, are reduced to 1) identification of positive results of their own pedagogical practices with a pronounced empirical and descriptive dominant, 2) superficial explanation of what to consider as management in educational organizations (while, as a rule, authors focus only on one part of the educational process – a school or university), and 3) clarification of the meaning of categories and concepts related with management issues in education, designing tables, exemplary plans, charts, diagrams, etc.

Management in the educational system in the papers of some modern authors acts as: a) purposeful, systematically organized process of influences on its structural components and the connections between them, ensuring their integrity and effective implementation of functions, its optimal development [5]; b) scientifically-organized management with a peculiar hierarchy: the first level –

the management of the activities of the teaching staff, the second level – the management of students [6]; c) a set of principles, methods, organizational forms and technological methods of teaching pedagogical systems, aimed at increasing the efficiency of their functioning and development [7].

Analyzed literary sources related to the management of education, can be divided into three groups: the first group encompasses the management of the educational process (some authors focus on training, education, or personality development); the second one deals with the management of educational organizations; the third group is related to the management of systems (programs, projects) of education.

Management in the field of education is a specific branch of managerial sciences that has absorbed the origins of pedagogy, psychology, sociology of management, management and marketing. Educational management has its own specifics and inherent regularities. Professional knowledge of management leads to the use of three different managerial tools by education managers:

1) organization, hierarchy of management, with the main focus on the impact on the person from above, with the help of motivation, planning, organization, control, stimulation, etc.

2) culture of management, that is a set of values, developed and recognized by society, organization, group of people, social norms and attitudes, features of behavior;

3) market, market relations, that is, relations based on the balance of interests of the seller and the buyer [8].

The key to understanding the essence of education management is the term "praxeology". Analyzing the meaning of this concept, we turn to the historical aspect of the emergence of praxeology.

The term "praxeology" was first used in 1897 by A. Espinas in the book Les Origines de la Technogie, the collection of his works, published in various philosophical journals of the 1890s. A. Espinas wrote: "A craftsman crafts, a peasant plows, a sailor steers a ship, a soldier fights, a merchant trades, a professor teaches, a manager manage. And then, the word "practice" generates the term "praxeology" for the definition of science about similar facts considered in their unity, the science of the most common forms and principles in the world of living beings" [9].

The Austrian economist L. Mises, in his work "Human action" (1949), used the term "praxeology" to refer to the science of human behavior. Being a representative of the Austrian Economic School, in the middle of the XX century, he pointed to the need of creating a general science studying human activity – praxeology (which literally means "logic of action") and emphasized the interdisciplinary nature of the study of human activity, the need of the integration of social sciences into general meta-science [10].

In the middle of the XX century, the great Polish philosopher and logic T. Kotarbiński defined praxeology as a general theory of effective organization of activity. He noted that praxeology covers three groups of problems: analytical description, characteristics, classification and systematization of practical actions; study of the conditions and laws that determine the effectiveness of action; study of the genesis and development of various types of activity, ways of their improvement and regression, moving forces of tradition and ingenuity. According to T. Kotarbiński, praxeology is a discipline that synthesizes the data of various sciences related to the organization of work. It combines only what can be applied to any activity and has a general character. Praxeology is a typical model of organizational science with a tendency to develop practical advice, with the assimilation of data from dozens of sciences, with the systematization of these data from the perspective of the tasks of this sphere of activity [11].

The analysis of scientific and educational works shows that the term "praxeology" is used in various contexts: praxeology of family relations, philosophical and legal praxeology, law praxeology, political praxeology, reflexive praxeology, pedagogical praxeology, and others.

In pedagogy, the praxeological methodology became relevant in connection with the increasing attention to rational and productive pedagogical activity in the context of the complication of pedagogical work.

Pedagogical praxeology in the system of professional education acts as a general methodology, which accumulates regulariies, categories, laws (by which the phenomenon of professional activity is explained), general scientific approaches related to the consideration and solution of problems of professional activity of a person and the laws of construction of their activities, interdisciplinary knowledge, which is a cumulative theoretical and practical experience of rational implementation of professional activities; the results of reflection on the causes, conditions and mechanisms of success of the activity.

The notion of management culture is in close relationship with the concept of praxeology. Culture of management is a set of achievements in the field of knowledge and scientific outlook, which reflects the moral and ethical norms of work and attitude to work. As any culture, it includes the relationship between material and spiritual, and it should also be considered from the standpoint of the unity and integrity of the culture itself. Management culture is defined as an integrated, generalized indicator of managerial work, which reveals its qualitative features and peculiarities. The education management culture represents the system of influence of the legal and socio-political centers of decision-making on the sphere of education, the system of mobilization and attraction of means for its effective production, improvement and modernization taking into account the established positions of the educational-scientific-pedagogical community in various fields of state activity.

The main peculiarity of the management of the system of higher education in modern conditions is that relevant problems have been solved in recent years only at the level of the educational system. The most important principle of managing higher education at all levels of the United States is the principle of systematicity. The effectiveness and success of a higher educational institution depends on the

success of its strategy in the market of educational services. Higher education management can be considered effective if it provides high quality education for the successful economic and social policies of universities.

The basis of management in the system of higher professional education in the United States is the integrity of different levels of educational management. Therefore, we will represent management as a process of influence, and educational management as a management system [12]. The effectiveness of such management system depends on the degree of interconnection of its levels and their continuity. Assessment of managerial decisions at all levels is embodied in the efficiency and success of university management.

Managing the University in the United States under current conditions requires the involvement of various social institutions, mainly families, in addressing educational issues, as well as the role of students in organizing and supporting the learning process.

The role of the student is determined by the independent career choice in a fairly differentiated educational space, as well as by the fact that they participate in the payment of their studies. A family in the organization of higher education plays a significant role, too: a) if family is able to pay for education, it participates in its financing; b) taking part in the choice of a higher educational institution, as well as specialty of an applicant, it has a certain impact on the content of education; c) participating in distance education on the basis of telecommunications, family contributes to the creation and development of an efficient learning environment [13].

One of the peculiarities of the management of the modern educational system in the United States is the development of the autonomy of educational institutions, the ability to independently determine the main characteristics of the educational process, in particular, teaching methods and technologies, the personnel potential structure, funding sources, the contingent of students, the overall ability to ensure the quality of the educational process.

Thus, the main peculiarities of the management of the modern educational system in the United States, which determine its strategic orientations, are the following:

1. The need for active policy of the development and modernization of the system of higher professional education, first of all at the national level.

2. The basic principle of the management of higher education at all structural levels (at the state, regional, municipal, and also at the level of higher educational establishments) should be recognized as a systemic principle.

3. Management in the system of higher professional education must meet the needs of society and ensure the availability of education.

4. The effectiveness of the management of the system of higher professional education should be ensured through the division of competences of organizations of different vertical structural levels in relation to the development of the principle of autonomy of universities concerning the organization, provision and management of the educational process (the choice of teaching methods and educational technologies, structure of employed teaching staff, sources of funding, contingent of students, etc.).

5. Development of healthy competition between higher educational institutions of different organizational and legal status.

6. Ensuring the quality of education and developing a system for its monitoring.

Recently, in Ukraine, there have been profound changes in all spheres of society. More and more attention is paid to the problems of professional education. The universities face the task of training not only a specialist who possesses the necessary knowledge, skills and abilities. In the context of modern trends in the development of production, labour market requires specialists who are able to independently solve new professional tasks, achieve a high level of success in activities possessing the necessary professional activities for a complex of professionally significant qualities.

In addition, at the present stage of development of Ukrainian higher education, the attitude of young people to education and work is undergoing positive changes. Modern students are better oriented at the labor market and more clearly understand what the employer expects from them. The orientations of Western students are gradually being passed on to Ukrainian peers: more attention was paid to success, social activity and community work. Many universities introduce rating systems, and Ukrainian employers, like Western ones, at the stage of reviewing the resume want to get an idea of the educational success of a former student. Despite this, so far the market of Ukrainian young professionals can not be called mature. Right now, at the time of the formation of more stringent and strict requirements for graduates, the differences between the Ukrainian students and students of the West, in particular, the USA, are well traced. With the development of the labor market for young professionals, this difference will be gradually eroded. This is especially important for the field of medicine, on which society's standard of living depends to a large extent.

The system of values, orientations and motives has repeatedly become the subject of research by Ukrainian and foreign scholars. Among recent empirical studies a special attention should be paid to the work of American scientists Ch. Brazeau, R. Schroeder, S. Rovi, L. Boyd [14]. They explore the relationship between such qualities and phenomena as empathy, professionalism and professional burnout. American researchers J. Rhoads, J. Gallemore, D. Gianturco, S. Osterhout made a comprehensive study of the relationship between students' motivation, their enrolment in university and academic achievement [15]. Comparative studies of values and attitudes of students in different countries are less numerous. One of the recent studies dealing with this problem is a comparative study of the differences in the responses to stressful situations between American and Canadian students performed by L. Dyrbye, M. Thomas, T. Shanafelt [16] and a comparative study of life satisfaction level of American and Russian students conducted by K. Tucker, D. Ozer and

S. Lyubomirsky [17]. However, an attempt to explore the differences in the area of professionally significant motives and values between Ukrainian and American students has not yet been implemented.

To study these differences, a survey of motivational orientation of Ukrainian and American students, their willingness to learn and work, basic persuasions, essential for successful learning and working activities was conducted.

THE AIM

To analyze the problem of university management in the cultural and philosophical aspects.

MATERIALS AND METHODS

The research was conducted in the form of a written questionnaire. Students were supposed to give a single answer to each question. The survey was conducted in September 2017-2018 and 2018-2019 academic year at Ternopil National Medical University. It encompassed 274 second-year Ukrainian students of medical, dental and pharmaceutical faculties and Institute of Nursing and 228 first, second and third year foreign students, who have come from the USA and study at the faculty of foreign students and International School of Medicine. Although the number of foreign students who participated in the survey was small, we believe that the number of respondents was sufficient enough to draw conclusions about the general trends in the issue under study.

Leading Ukrainian specialists in the field of philosophy of management focus on the psychological aspects of management activity, which becomes of particular importance "due to the significant increase in the role of the personal factor in ensuring the desired efficiency of production and the joint activity of people in general, due to the nature of post-industrial society". It is also combined with the necessity of forming a high managerial culture of the head and skills of interpersonal and, in particular, managerial communication, and with the implantation and the proper skills to work effectively in conditions of information uncertainty and increase of the level of risk. [18]. One of the important factors of effective management by university leaders is information support, operational analytical information on the motives for choosing an educational institution, adaptation in the new environment, adherence to academic traditions, values system.

Within our research, following I. Feduh, we will understand value orientations as the general regulators of human behavior, which reflect the life experience gained by the individual in individual development, and is the core of consciousness, taking into account the person's decision on important life issues [18]. Professionally meaningful value orientations will be understood as a set of attitudes, assessments and motives that underlie the formation of meaningful and chronologically agreed professional goals and plans [18]. This will include the motives for choosing a profession and enhancement of academic performance, attitudes to offenses in the course of educational activities, assessment of the existing system of training.

RESULTS

Analysis of responses given by Ukrainian and American students allowed us to single out certain trends and specific differences in their outlook and professionally relevant persuasions and attitudes. A positive peculiarity is that the main motive that prompted Ukrainian students to choose a medical profession was that they believe it to be humane and noble (36%). Despite this, many students consider other important criteria when choosing a profession; particularly that it is prestigious and well-paid (21 % and 13 % respectively). The negative sign is that relatively big amount of students in the comparison with Western countries (the USA in particular), indicate that the occupational choice was influenced by their parents' desire (14 %).

Unlike the Ukrainian students, their American peers are largely guided by the fact that medical profession is a well-paid job (37 %). Many students also (36 %) decided to become doctors as long as this is a caring, humane and prestigious (24 %) profession. Only 3 % of students decided on medicine because it was the desire of their parents.

The main difference between Ukrainian and Western students is the latter's more pragmatic approach to acquired knowledge and considering them the foundation for future career. In Ukraine, many students perceive their university years as carefree period when there are few limitations and you can disobey the academic rules without any punishment. In the USA, the phenomenon of cheating does not exist which is proved by the answers of the students: only three students chose the variants justifying cheating.

At the same time, the overwhelming majority of Ukrainian students justify cheating by the lack of time for preparation (38 %). In addition, a large number of students consider cheating to be useful as it is, in their opinion, facilitates memorization of learning material (30 %). A considerably smaller number of students disapprove of cheating as an unacceptable phenomenon.

Foreign students while answering this question have demonstrated the opposite attitude. Thus, most students (45% and 40%) treat cheating quite negatively, and only 24 students out of 228 justify it.

Answering the question «What do you think about your university life?», the majority of Ukrainian students chose the answer «During this period of time, I will obtain new profession and new acquaintances and experience» (37 %) and «It is a difficult period when I will have to work hard to become a good specialist» (28 %). Interestingly, the relatively large number of students still believes that this will be a carefree period of their lives (15 %). At the same time, most American students consider this period as a serious stage for their career: 62 % think that this will be a hard time requiring diligent work and only 10 % recognized it as a carefree stage of their lives.

Another positive peculiarity is that, according to many students, university education will fully prepare them for

a future profession (43 %). At the same time, one must pay attention to the fact that many students believe that despite the fact that the university will give them a deep theoretical knowledge, the actual acquisition of practical skills will begin with the start of their professional activity (41 %). A relatively large number of students (16 %) chose the option «University education does not give you enough knowledge for future work and after you get your first job you will need to learn everything on you own again», which is a negative sign, which universities should strive to overcome.

Foreign students' answers were significantly different. The majority believes that the university will equip them with deep theoretical knowledge whereas the acquisition of practical skills will begin with the start of professional career (52 %). Interestingly, none of the American students chose the variant «University education does not give you enough knowledge for future work and after you get your first job you will need to learn everything on you own again».

Most Ukrainian and American students believe that higher education is the key to successful future (59 % and 76 % respectively). However, according to many Ukrainian students, higher education is a pure formality (32 %). The negative sign is that about 16 % of Ukrainian students believe that obtaining higher education is a waste time. Interestingly, none of the foreign students chose this option.

Most respondents believed that high quality of higher education is the key to easy employment after graduation (57 % and 85 % respectively). Relatively large number of Ukrainian students (20 %) suggests that they will need to resort to corruption while searching for the job. However, no foreign student chose this option. Interestingly, much more Ukrainian students admit the possibility of retraining in the future than their American peers (23 % and 15 % respectively).

Assessing the state of higher medical education in Ukraine, many students (44 %) recognize the need to borrow foreign experience of higher medical education organization. Moreover, half of the Ukrainian (47 %) noted the need to adapt international experience to the educational conditions of Ukraine. One third of respondents (39 % and 26 %) believe that the experience of Germany and the United States concerning the organization of higher medical education is most valuable. Interestingly, that in this category Ukraine takes the third place.

The answers of the American students were somehow different. The overwhelming majority of students (77 %) believe that the highest level of higher medical education is in the USA. Fewer respondents chose Germany, Canada and Austria.

CONCLUSIONS

As conclusions, it is expedient to draw attention to the following aspects.

An important prerequisite of increasing the level of management activity in a modern university is the level of teachers' philosophic culture. This requires special attention to the process of professional selection and advanced training. This factor causes insufficient attention to the definition of the system of basic concepts, the search for the laws of human development to the level of personality.

On September 12, 2018, the Ministry of Health of Ukraine presented the "Strategy for the Development of Medical Education in Ukraine". This peculiar document provides a number of interesting innovations. Among the drawbacks is the lack of a methodology for assessing the needs for training health care workers, reducing the prestige of the profession of a doctor, as a consequence – the lack of motivation to study medicine, lack of trust in higher medical education institutions, medical universities do not fulfill the functions of public education in issues related to health. Authors of the document prove that the content of education needs to be updated. Knowledge received by students is not always relevant and modern, the gap between higher education, science and practical activity as well as the lack of funding for research, university hospitals, clinical facilities for practical training of students are still noticeable. Of particular interest is the requirement to change the academic culture, as the medical university / faculty "should be the place of a free open discussion in order to create new ideas and seek new knowledge". Obviously, these problems are important for all specialties.

Each science at a certain level of its development is approaching the establishment of laws and regularities. Their search at the level of philosophy of education, philosophy of didactics is promising and important. This aspect is one of the main issues in the development of the teacher's didactic culture. Without understanding the laws of the development of science, including the regularities of teaching and learning, we will have a chaotic accumulation of information.

Information support of the educational process is an obvious necessity. In some higher educational institutions, laboratories for monitoring education quality have become widely distributed. Systemic collection of information, use of sociological techniques allow the modern manager to take effective managerial decisions, analyze the problems of professional choice, adaptation, expediency of the undertaken measures, factors that determine the authority of teachers and departments.

Every year, the number of foreign students in universities is increasing, as well as the number of young Ukrainians who are studying in other countries. Readiness to learn in a multicultural environment is a new challenge that science and practice need to respond to.

At the time, publishing new generation of manuals on pedagogy of higher education is of paramount importance. Modern science is impossible without integration of information flows. An important place will be taken by the experience of past years.

The Internet system allows solving individual didactic tasks in a new way. Thus, our lecture, devoted to the regularities in pedagogy and the system of basic concepts, was watched by more than 13 thousand users over a short peri-

od of time. University libraries can collect the best lectures, practical classes, techniques, and reflections by leading scientists on important issues of theory and practice.

In the context of the problem under study, let us single out the issue of development of the culture of mental activity. This topic will be the subject of several doctoral dissertations. After all, such issues as pedagogical regularities of successful teaching and learning, the development of professional creativity, self-improvement as a factor in personal development, fatigue prevention, the question as an element of the didactic system, author's didactic and methodical systems do not lose relevance.

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REVIEW ARTICLE

VESICAL IMAGING-REPORTING AND DATA SYSTEM – A NEW APPROACH TO BLADDER CANCER STAGING

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ABSTRACT

The aim: To present the assumptions and to show the usefulness of Vesical Imaging-Reporting and Data System (VI-RADS) based on multiparametric magnetic resonance imaging (mpMRI) in the diagnostic pathway management of patients with a bladder cancer.

Materials and methods: The review is based on available literature from last 10 years from PubMed database and the *Przegląd Urologiczny* journal focusing on articles on VI-RADS. Overall, 18 articles were included. Presented magnetic resonance images come from the examinations of the patients who were diagnosed with bladder cancer from 2019 to 2021 at Department of Diagnostic Imaging in University Clinical Hospital in Opole, Poland.

Conclusions: The newly developed Vesical Imaging-Reporting and Data System has a potential to play a significant role in staging of the bladder cancer as a non-invasive, comprehensive, and effective diagnostic tool providing accurate information for differentiation non-muscle-invasive bladder cancer (NMIBC) from muscle-invasive bladder cancer (MIBC). However more prospective studies should be conducted to validate this system in clinical practice.

KEY WORDS: neoplasm staging, urinary bladder neoplasms, multiparametric magnetic resonance imaging, reference standards, diffusion-weighted magnetic resonance imaging

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INTRODUCTION

Bladder cancer (BC) is listed as 9th most common cancer [1] and ranked 13th considering its lethality on a global scale [2]. Because of its prevalence, as well as significant morbidity and high recurrence rate BC has one of the highest lifetime treatment costs among all cancers [3]. Estimated numbers of 430,000 new BCs diagnosed annually and 165,000 resulting in death worldwide [2] show the scale of the problem. Most common risk factors are cigarette smoking, exposure to aryl amines and radiation as well as long-term use of some drugs. Majority of BCs are of urothelial origin with less common squamous cell carcinomas, adenocarcinomas, and small cell carcinomas [4]. Nowadays the recognition and staging of BC is based on histopathological examination of a specimen taken during transurethral resection of bladder tumour (TURBT). Definition of the depth of the bladder wall infiltration is essential for the further management. Despite TURBT remains a gold standard for local staging, it is prone to omitting muscle infiltration in up to 25% of cases, which can lead to understaging resulting in delayed or inadequate treatment [5]. Additional consideration of the results of sectional imaging may reduce the risk of diagnostic errors, especially when using a method providing high tissue and spatial contrast, such as magnetic resonance imaging (MRI). Vesical Imaging-Reporting and Data System (VI-RADS) based on mpMRI has been developed in 2018 to standardize acquisition and interpretation of diagnostic images in BC as a non-invasive substitute of TURBT in staging [6].

THE AIM

The purpose of this review is to present general principles and potential value of VI-RADS as a newly developed tool in the diagnostic process and management of BC based on available literature.

MATERIALS AND METHODS

Articles retrieved from PubMed database were surveyed to create this review, employing following key phrases: "VIRADS", "Vesicle Imaging-Reporting and Data System", "multiparametric magnetic resonance imaging" and "bladder cancer". 18 of founded objects publicised in last 10 years were considered for analysis. With the same searching strategy 2 studies from the *Przegląd Urologiczny* journal were chosen.

The magnetic resonance images presented in this review are obtained from the diagnostic procedures performed on patients of the University Clinical Hospital in Opole. All of them were diagnosed due to BC from 2019 to 2021.

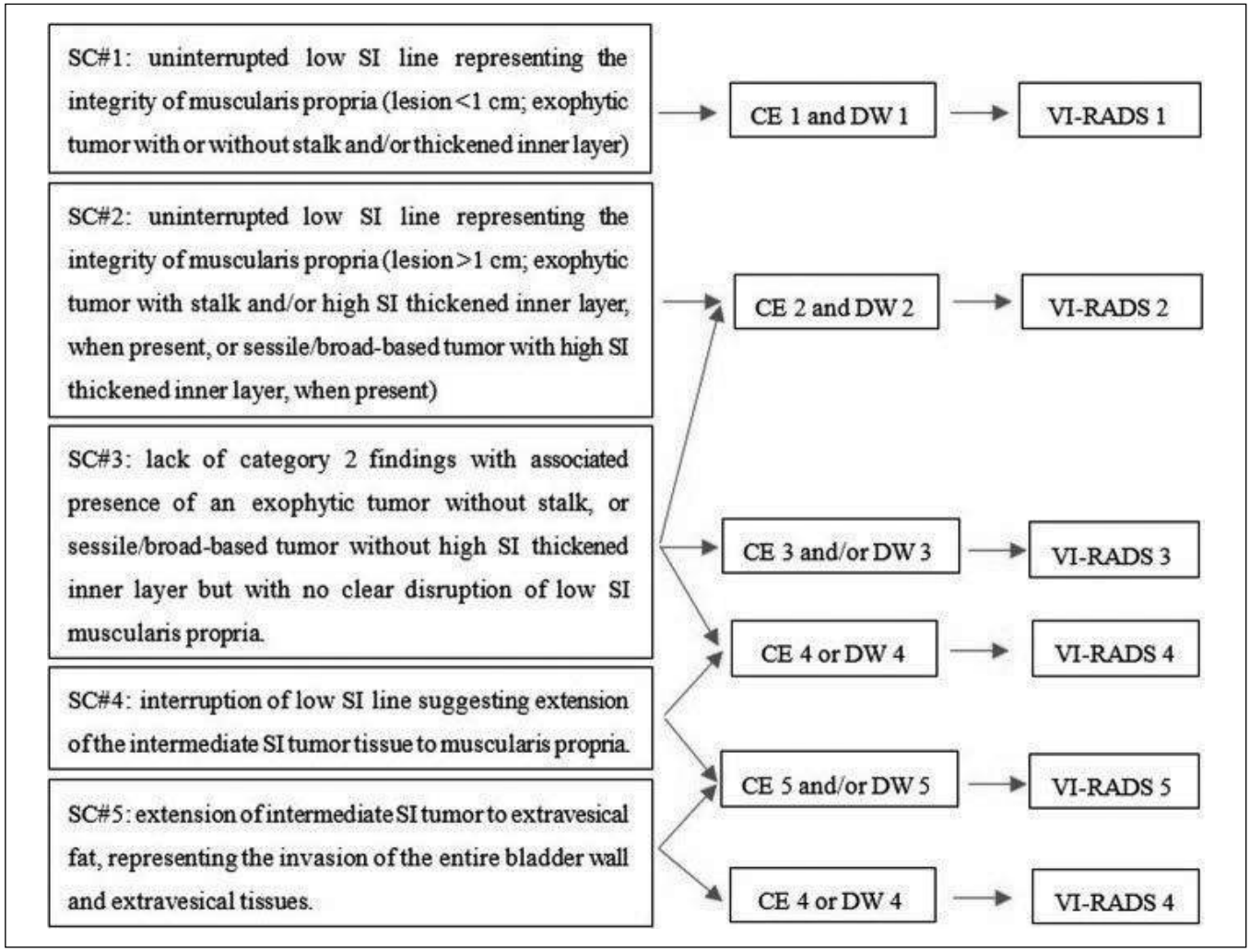

Fig. 1. VI-RADS scoring rule schematic. CE = contrast enhanced; DW = diffusion weighted; SC = structural category; SI = signal intensity Figure from original publication (Evaluation of the value of the VI-RADS scoring system in assessing muscle infiltration by bladder cancer) [1].

REVIEW AND DISCUSSION

THE DEVELOPMENT OF THE VESICAL IMAGING-REPORTING AND DATA SYSTEM (VI-RADS)

Vesical Imaging-Reporting and Data System (VI-RADS) with the five points scoring scheme were introduced by Panebianco et al. [6] and it was inspired by Prostate Imaging-Reporting and Data System. The main goal of VI-RADS is to evaluate the local staging of BC, by estimation of the probability of bladder wall muscle infiltration before performing a surgical procedure [7]. Based on detrusor muscle involvement, tumours lesions can be divided into non-muscle-invasive bladder cancers (NMIBCs) and muscle-invasive bladder cancers (MIBCs). In principle, NMIBCs are less aggressive and have much better prognosis than MIBCs, so these types of lesions are treated differently. As TURBT can understage even up to 45 percent of BC causes when resection unintentionally does not include detrusor muscle layer [7], there is a necessity to develop an alternative diagnostic procedure not dependent on operator's skills. Magnetic resonance imaging (MRI) is a valuable tool in clinical practice due to its high tissue contrast and the lack of radiation exposure [8, 9]. The VI-RADS scoring scheme's aim is to standardise the evaluation based on multiparametric magnetic resonance imaging (mpMRI) [6, 10].

PATIENT'S PREPARATION AND IMAGING

A proper bladder distension is vital for optimal visualisation of its wall layered structure, including detrusor muscle. It can be achieved by instructing patient to void 1-2 h before imaging and then to drink 500-1000 ml of water half an hour before an examination [11–13]. Contracted and thick wall of the bladder during MRI procedure may result in over staging of the tumour, while its overdistension may induce motion artifacts and patience's discomfort [11]. To reduce bowel peristalsis artifacts, it is adequate to administrate antispasmodic agent [12].

VI-RADS provides standardized reporting criteria on mpMRI and was validated using 1.5T and 3T MRI scanners [6–9, 11–16], however 3T scanners offer better sensitivity and specificity when mpMRI is considered [16]. There are

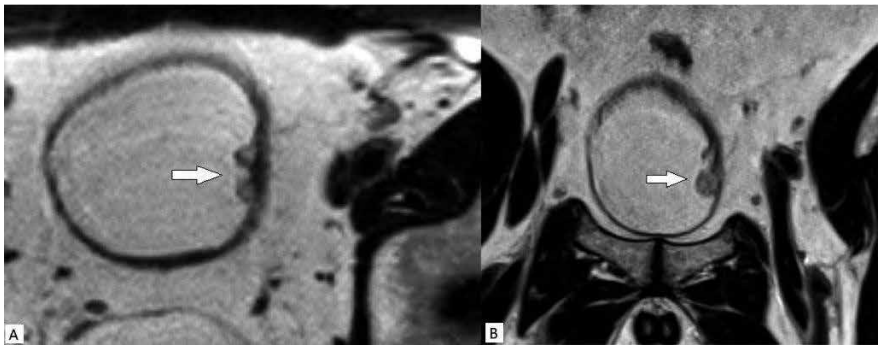

Fig. 2. MRI T2-weighted imaging in transverse (a) and coronal (b) plane reveals the bifocal exophytic hyperintense tumour without interruption of the low signal line of the muscle layer – VI-RADS 1.

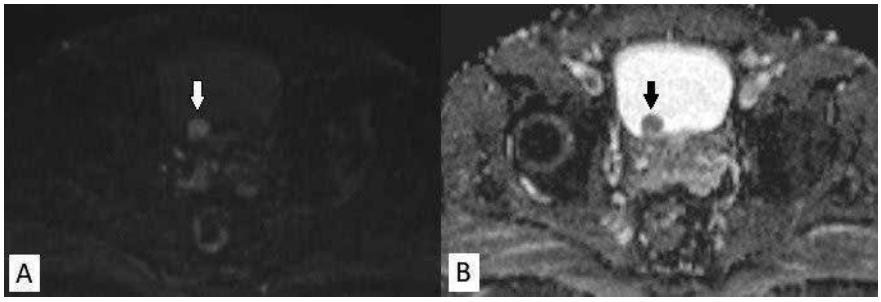

Fig. 3. MR images in DWI (a) and ADC (b) sequences show a tumour of the posterior wall of the bladder. Within this lesion a high signal in DWI and a low signal in ADC images was detected, indicating a restriction of water molecules diffusion, with the correct signal of the adjacent muscle layer maintained – VI-RADS 2.

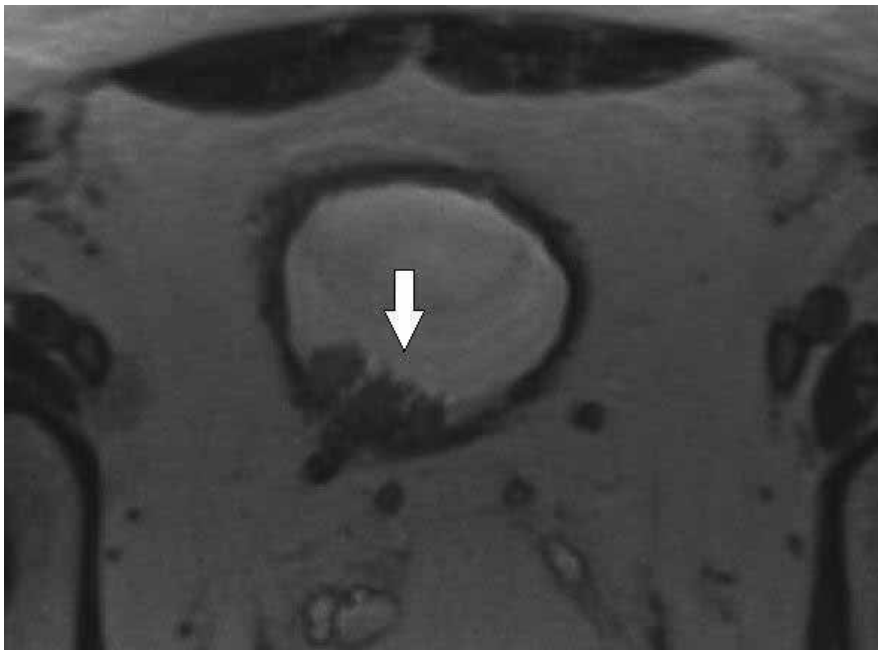

Fig. 4. T2-weighted transverse image demonstrates a tumour of the posterior-right lateral wall of the bladder with an interruption of a muscle layer low-signal line continuity – VI-RADS 4.

three main imaging sequences that are applied to evaluate the VI-RADS score: T2-weighted (T2W), diffusion-weighted (DWI) and dynamic contrast enhanced (DCE) [6, 12, 14]. Normal bladder mucosa is not visible on T2W and DWI sequences but reveals early phase enhancement in DCE imaging. Muscularis propria shapes a hypointense line on T2W images, without any enhancement in DCE imaging. An interruption of its signal continuity may suggest the infiltration of the tumour into the wall's muscle layer. After contrast agent administration BC tissue along with the mucosa reveal an early enhancement while on DWI it is hyperintense [6]. Several studies also indicated apparent diffusion coefficient (ADC), which allows for quantitively assessment of the impedance of water molecules diffusion, as the optional parameter to be taken into consideration, however there is no cut-off value that has been accepted [11,13].

SCORING

It is generally thought that small tumours are less invasive than bigger ones, but the contact area of the tumour with the bladder wall may also play a role in assessing a probability of the muscle infiltration. However, these premises are not always certain and require verification [5]. Facing these doubts, Panebianco et al. [6] developed a scoring scheme (Fig.1).

The score is an overall estimation generated by the assessment of tumours in T2-weighted, DWI and DCE imaging

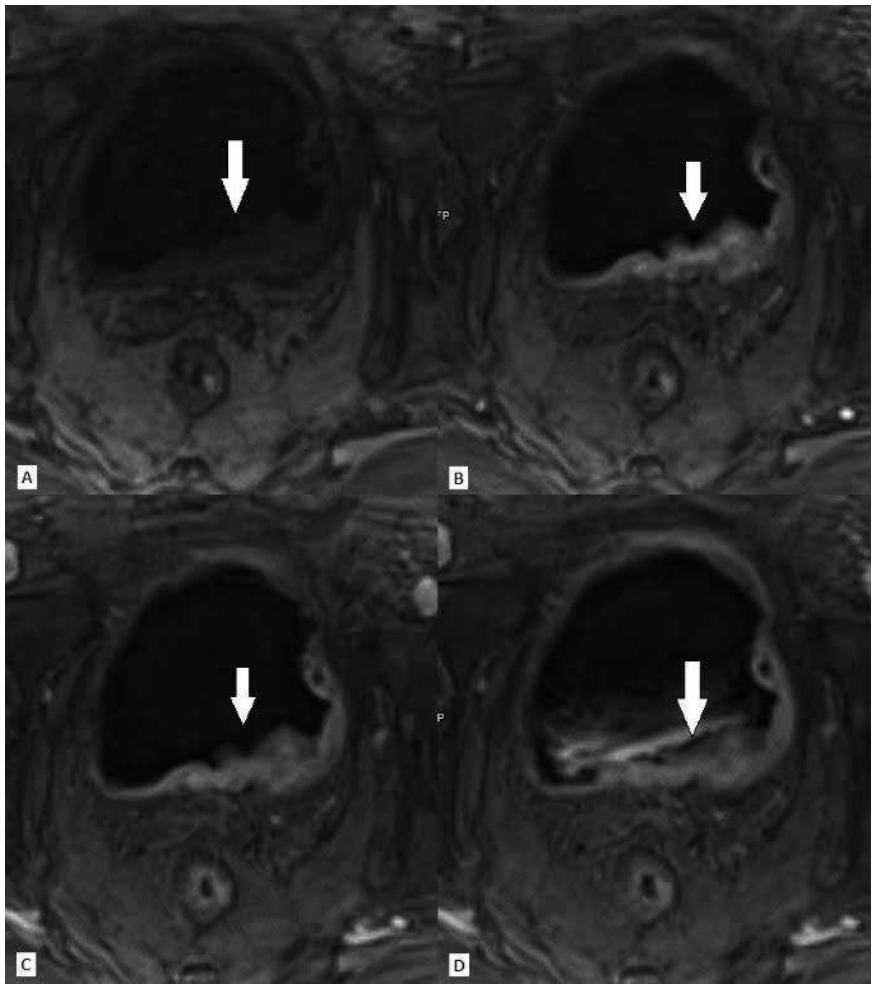

Fig. 5. Subsequent (a-d) T1-weighted transverse images with fat saturation after administration of the contrast agent (DCE) revealed an early enhancement of the extensive tumour infiltrating the posterior-left lateral wall of the bladder and the perivesical fat tissue – VI-RADS 5.

[6, 12, 14]. The five-point scale is helpful in the evaluation of the muscle invasion, to be present [5, 6]:

- VI-RADS 1 – highly unlikely (Fig. 2),
- VI-RADS 2 – unlikely (Fig. 3),
- VI-RADS 3 – inconclusive,
- VI-RADS 4 – likely (Fig. 4),
- VI-RADS 5 – highly likely (invasion beyond the bladder is probable) (Fig. 5).

Nowadays mpMRI is believed to be one of the most promising tool to evaluate the staging of the BCs, therefore it might play a crucial role in pre-TURBT and preoperative procedures [6–9, 14]. Standardization of MRI, which ended up creating VI-RADS, provides high diagnostic precision and accuracy regarding that VI-RADS 3 has better sensitivity while VI-RADS 4 and 5 have better specificity [1, 5–7, 10, 14, 17]. During invasive diagnostic procedures, like remaining gold standard – TURBT, many lesions might be potentially under staged or there is a missing detrusor, while those issues do not appear when mpMRI is implied [1, 6, 14]. As an operator-independent method MRI also reduces the likelihood of missing multiple lesions compared to flexible cystoscopy [9]. Hight diagnostic accuracy provided by standardised mpMRI approach enables to reliably differentiate NMICB and MIBC, resulting in clinical management [1, 6, 11, 15, 18]. Wang et al. [15] reported 100% accuracy of muscle invasion prediction in case of lesions scored as VI-RADS 1, 4 and 5 and approximately 95% accuracy in VI-RADS 2, with lower predictive value in case of VI-RADS 3 tumours. Marchioni et al. [19] suggested VI-RADS 4 as the best cut-off value. Establishment of VI-RADS scale did not significantly improve the precision of MRI itself, but standardized and unified results interpretation [17]. Furthermore, it has been proven that the intra- and interobserver variability of VI-RADS scoring is very low and most radiologists agree on the score [1, 7, 15, 17]. The agreement within and between the groups of experienced and inexperienced readers was substantial while it was greater considering experienced ones [20]. Ueno et al. [20] concluded that patient diagnosed with score of 1 should aim for complete resection while the treatment those with the scores of 4 and 5 should be cytopathologic diagnosis as a confirmation rather than complete resection. Clinicians value this tool as it reduces time between proper diagnosis and treatment [19]. There are a few studies evaluating VI-RADS usefulness in monitoring BC treatment response and post-treatment follow-up as a non-invasive alternative for cystoscopy. Both in the case of differential diagnosis between tumour recurrence and post-treatment inflammation or fibrosis, as well in case of assessing the response to induction therapy, superiority of DWI over T2W and CE sequences were observed [6]. These results, although promising, require further validation and

prospective clinical trials to be considered as an alternative to re-TURB [14].

There are some limitations of VI-RADS scoring scale that need mentioning. Difficulty in maintaining the bladder filled may reduce the precision of distinguishing between VI-RADS 3 and VI-RADS 4 [9] and over-filled bladder, on the other hand may result in motion artifacts distorting images [6, 9]. Scoring lesions located in the bladder neck or ureteral orifices may be also challenging. Some benign pathologies like, e.g. inflammation or fibrillation of bladder wall and post-treatment changes may lead to over staging and misdiagnosis [1, 6, 13, 17]. The probability of inaccuracy in these cases is reduced by a combined assessing of all three sequences included in the scoring. Despite of its modality high tissue resolution some pathologies, carcinoma in situ, e.g., cannot be diagnosed using mpMRI with a satisfactory accuracy [12]. Clinicians suggested that BCs located in the bladder neck and bilateral ureteral orifices may draw difficulties with the assessment but the reason is unknown [15, 20]. What is more, to take the personal approach on the treatment the histopathological assessment is still needed because VI-RADS scale does not inform about tumour's biology [12]. The study of Ahn et al. [5] proved that clinical surveillance is necessary, as in some VI-RADS 2 cases the infiltration of the muscle was diagnosed eventually. Although mpMRI implementation as a diagnostic modality allows to avoid patient's radiation exposure but requires gadolinium contrast agent administration, which potentially endanger patients with renal insufficiency to nephrogenic systemic fibrosis. A solution in this matter may become the biparametric assessment based on T2W and DWI images, similar to those proposed in PI-RADS.

In conclusion, today TURBT remains urooncologists' first choice for diagnosis of BCs but VI-RADS scoring scale has a potential to partially replace invasive diagnostic methods in recognition of muscle invasive BC and to become a valuable tool in treatment management and clinical follow-up, however its sensitivity, specificity and diagnostic value requires validation in further prospective clinical trials.

CONCLUSIONS

VI-RADS scoring scale comes to a light as promising and effective tool to evaluate bladder cancers local staging for more accurate clinical decision making. Its application can potentially cause the diagnostic and treating process to be more reliable and effective, but also has a potential to reduce the invasive diagnostic procedures to the indispensable necessity. However, there is a necessity for more prospective studies to rate the value and usefulness of VI-RADS scale to be conducted.

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REVIEW ARTICLE

NATIONAL HEALTH FINANCING SYSTEMS AGAINST THE BACKDROP OF A GLOBAL PANDEMIC: NEW CHALLENGES AND PROSPECTS

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ABSTRACT

The aim: The purpose of the article is to study the challenges and prospects for the development of national health financing systems against the background of a pandemic.

Materials and methods: Our research consists of two interrelated stages. At the first stage, the central task was to determine the size of the drop in world GDP. In the second part of the study, we focused on the definition of the essence of concession agreements in order to use t in the field of medical care. The key methods used in this study were data analysis, generalization, and comparison.

Conclusions: National health financing systems in the vast majority of the world's countries have experienced unprecedented pressures and problems both in terms of health insurance due to the huge losses of the insurance industry in 2020. In order to improve health care financing systems, it is necessary to update the forms, methods and tools of the insurance market functioning in terms of the health insurance segment, and to introduce new mechanisms for financing the medical sector in the process of combating the spread of coronavirus infection.

KEY WORDS: global pandemic, coronavirus disease, healthcare financing systems, health insurance, concession agreement

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INTRODUCTION

In March 2020, the World Health Organization officially recognized the spread of an infectious disease caused by SARS-CoV-2 as a pandemic, which is characterized by an extremely high level of population damage. The coronavirus pandemic has created an unprecedented global public health emergency and identified existing defects in the medical systems of all nation states, and in underdeveloped countries demonstrated a catastrophic state of health care in terms of ensuring the availability and quality of health care against the background of the lack of an effective health financing system.

The most urgent need to solve problems appeared before the countries, the system of financing the medical industry of which required fundamental transformations even before the start of the pandemic, although absolutely all states, even those that occupy the first places in the ranking of the most developed economies in the world, to one degree or another faced a situation of insufficient the safety margin of the health financing system in the conditions of a sharp increase in the load and a tough struggle.

LITERATURE REVIEW

Given the suddenness of the global pandemic and the speed of its spread, scientific research, which focuses on the socio - economic consequences of the pandemic, and, in particular, the problems of health financing in nation states, are in their early stages. The basis for determining the directions of medicine financing modernization is the research of global insurance corporations, scientific developments of domestic and foreign scientists regarding models of the health care system in different countries. (scientific works of S. Thomson, M. Litvinenko, K. Bendukidze, I. Kinash, L. Savchuk), as well as the introduction of various forms of public-private partnership, in particular in the field of medicine (works of T. Bondareva, V. Muzhilovsky, A. Martyakova, I. Trikoz, K. Pavlyuk, A. Portnoy, I. Taranenko, N. Chaloy, E. Cherevikov and many others). However, despite the thoroughness of these studies and recognizing the significant contribution of scientists to the solution of these problems, it should be noted that in modern conditions, it is of particular relevance to determine promising vectors of health care financing that are suitable for fairly rapid practical implementation, taking into account the need to overcome the consequences of the coronavirus disease pandemic in all countries of the world.

THE AIM

The primary purpose of the article is to study the challenges and prospects for the development of national health financing systems against the background of a global pandemic.

Table I. Characteristics of pandemics

Pandemic	Year	Pathogen (virus)	Number of infected people	Number of deaths (global estimates)	Mortality rate
Spanish flu	1918 – 1919	A/H1N1	500 million.	50-100 million.	2 – 3
Asian flu	1956 – 1958	A/H2N2	No ratings available	1-4 million.	No data available
Hong Kong flu	1968 – 1969	A/H3N2	No ratings available	1-4 million.	No data available
SARS	2002 – 2003	SARS-CoV	8098	774	9.6
Swine flu	2009 – 2010	H1N1/09	200 million.	From 18,500 (laboratory-confirmed cases according to WHO) to 203,000 (estimated by The Lancet)	0.03
MERS	Since 2012	MERS-CoV	1,917 laboratory-detected cases in 17 countries (WHO data)	677	36
Ebola	2013 – 2016	Ebola, hemorrhagic fever	28616	11310	39.50
Seasonal flu	Annually	Mainly A / H3N2, A / H1N1 and B	340 million - 1 billion	250,000 – 500,000 annually	Each type of flu has its own coefficient
COVID-19	Since 2019	SARS-CoV-2	103,340,145 (as of 31.01.2021)	2,233,577 (as of 31.01.2021)	0.27

Source: formed by the authors based on: [2], [3], [4], [5], [6], [7].

MATERIALS AND METHODS

Our research consists of two interrelated stages. At the first stage, the central task was to determine the size of the drop in world GDP because of the global pandemic and the worldwide losses of the insurance industry caused by the global disasters. This was done in order to figure out the ability of budget financing and health insurance to provide an appropriate level of health care financing as its main sources in all countries worldwide. In the second part of the study, we focused on the definition of the essence of concession agreements in order to use t in the field of medical care. The key methods used in this study were data analysis, generalization, and comparison. Data synthesis and analysis were key elements of the additional value of the study, and helped to identify the main modernization vectors of national health care financing systems against the backdrop of the global pandemic.

REVIEW AND DISCUSSION

Of course, pandemics of various diseases are not a new phenomenon in the history of mankind (Table I). But it should be noted that it is in the context of globalization that the socio-economic consequences of epidemic processes in individual regions are very quickly transformed into global phenomena of an extremely negative nature, which are unfolding on a planetary scale.

Thus, according to experts of the World Bank, pandemics today can be a real shock to the world economy: a serious pandemic can cause economic losses of almost 5% of world GDP, or more than 3 trillion US dollars, while losses from a "weak" influenza pandemic (like the H1N1 virus of 2009) can cost about 0.5% of world GDP [1].

In the context of a pandemic, the health systems of national states have demonstrated a desire to increase significantly the level of endurance, operating capability, stress-resistance, the ability to instantly make managment decisions and use forceful decisions of state authorities. At the same time, medicine itself also strives for adaptability, flexibility and responsiveness to such changes.

The pandemic of the new coronavirus primarily directly affects the sphere of public health, and at the same time has become a large-scale challenge to the normal functioning of all sectors of life of any world power. The forced introduction of quarantine measures increases the damage in business activity, negatively affects the financial system, social production and etc. And the process of reformatting international relations within the supranational political and economic associations of individual states in the process of concluding contracts for the supply of a global public good - vaccines that have a proven high level of effectiveness, generally deserves a separate study.

In addition to focusing on the medical field, governments around the world are also forced to devote significant budgetary resources to confronting the dangers of rising unemployment, poverty and social differentiation. It is precisely because of the multi-vector budgetary policy that no nation state, even those that occupy the first places in the rankings of countries with the largest GDP per capita, can take on such a responsibility as to fully provide their

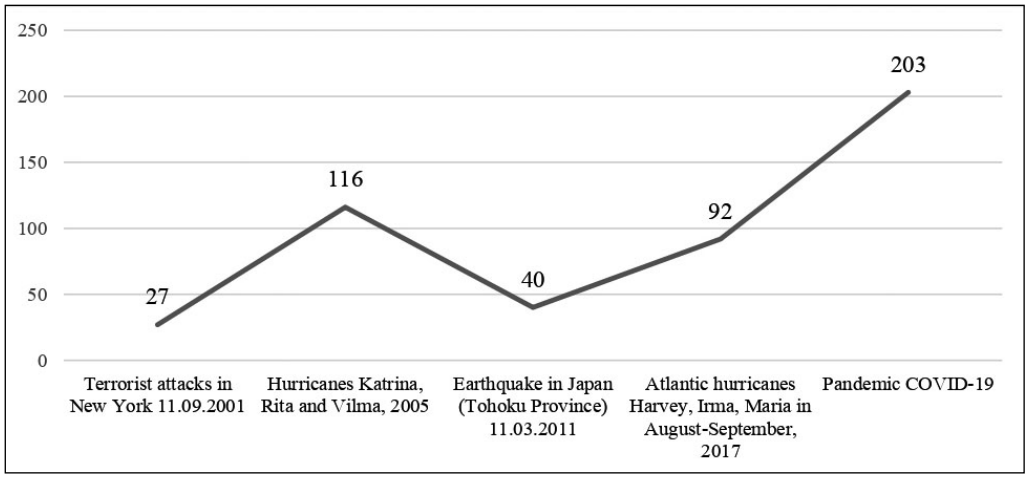

Fig. 1. Global losses of the insurance industry due to global disasters (USD billion)
Source: formed by authors on the basis of [10].

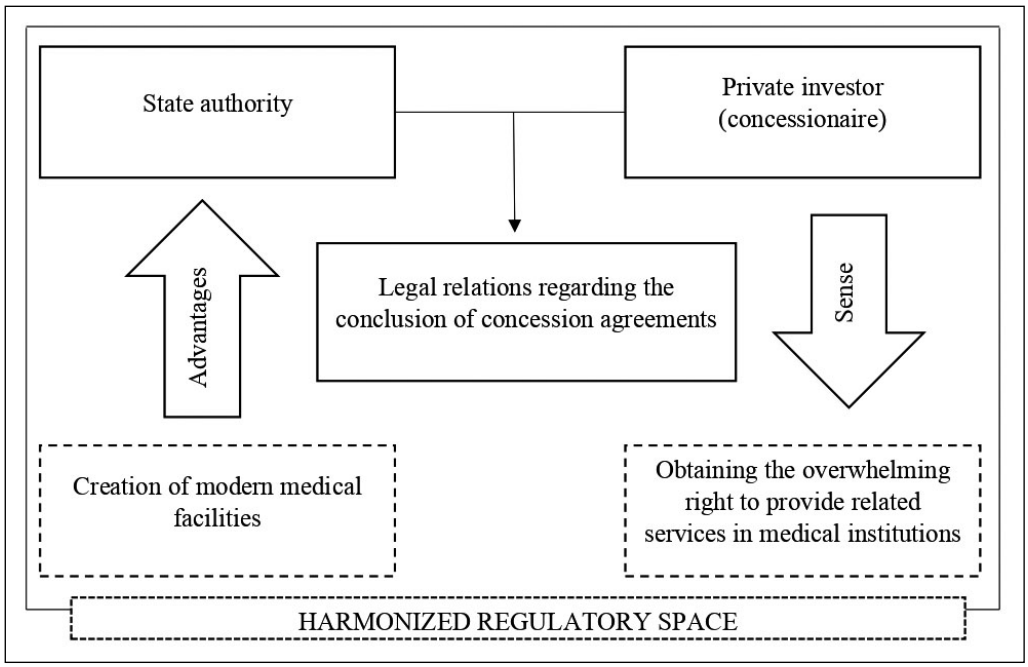

Fig. 2. Structural and logical scheme of interaction between public authorities and private investors in the process of implementing concession agreements in the medical field.
Source: formed by authors.

own citizens with free medical services.

The normative documents of the World Health Organization (WHO) define the following main sources of financing for medical services [8]:

- taxpayer funds (budget financing);
- voluntary health insurance of the population;
- employers' voluntary insurance programs (private sector spending);
- funds from donors or non-governmental organizations;
- direct expenses of individuals or households.

Models based solely on budget financing do not provide a reliable foundation for the dynamic development of national health systems in accordance with modern challenges and threats. It is absolutely clear that in any country the complex of medical guarantees in the absence of a health insurance system simply does not work.

The experience of the leading countries of the world shows that health insurance is the source of financing for the healthcare industry, which makes it possible to reduce the burden on the state and local budgets, as well as to create conditions for the most complete satisfaction of the needs of the population in affordable and high-quality medical care. It is also necessary to increase the flow of funds to the voluntary health insurance system, intensify the interest of medical institutions, employers and citizens in it by creating an optimal unified system of classification and pricing for medical services [9].

Medicine in modern conditions should mainly be focused on the ability to anticipate the likelihood of the development of certain processes, which, in turn, will improve the quality of medical services and create transparent conditions for the functioning of the health system. In modern civil society, the future belongs to high-quality insurance medicine.

At the same time, it should be noted that the coronavirus pandemic has become a difficult test for national health insurance systems (Figure 1).

According to information published by the leader of the global insurance industry, Lloyd's Corporation, the losses of insurers are now very high and will increase in the future.

The situation in the insurance industry is extremely unusual. Experts note that the amount of direct losses, which

consists of payments under health insurance contracts, life insurance, as well as from payments of insurance benefits in connection with the cancellation of corporate events due to the shutdown of business activities, etc., is an unprecedented amount of $ 107 billion. At the same time, losses from a decrease in the volume of investment resources due to a decrease in the value of assets are estimated at $ 96 billion. In general, these amounts represent the largest global losses in the history of the insurance business [11].

Currently, there are three main types of health care financing: state (budget), social insurance, and private health insurance. Of course, in practice, pure forms do not exist either in nature or in society. Therefore, the financing of health care is usually carried out in a mixed form. However, depending on which form of financing is dominant, the health care system is divided into state (England, Ireland, Italy, Scotland), with compulsory health insurance (Austria, Belgium, Netherlands, Germany, Sweden, Japan) and mixed (budgetary insurance) (USA), in which about 90% of the country's population use the services of private insurance companies [12].

There are such circumstances as: the limited budgetary resources in the overwhelming majority of national states, the lack of prospects for increasing financing of health care in developed countries, which are forced to overcome the entire range of socio-economic consequences of the pandemic, or in general, the critical level of underfunding of the medical sector in developing countries, the general failure of national health insurance systems due to unprecedented losses in the history of the insurance business. These abovementioned circumstances jeopardize the possibility of providing medical guarantees in any country, especially in the event of adverse events, such as: simultaneous vaccination of the population of a large number of countries in order to form herd immunity, constant mutation of the virus with even more severe consequences for health, insufficient level of effectiveness of the organizational measures of national states in the fight against the pandemic.

In today's world, characterized by the need for additional spending of budgetary funds on measures to overcome the socio-economic consequences of the pandemic, the budgetary system of any country is experiencing an additional burden, because budgets at all levels are becoming more and more limited, and therefore budgetary resources are not enough to finance all public goods. At the same time, it should be noted that if the state assumes the vast majority of responsibility for the health of the nation, employers and citizens themselves stop caring about it. That is why the most effective in modern conditions are health financing systems based on mixed budgetary and insurance models.

Therefore, this gives us reason to believe that in the modern environment, the modernization of the health care financing system at the level of each state should take place in two main directions: on the one hand, this is the development and support of a solvent and financially stable health insurance market, which should ensure the provision of a full complex medical services in accordance with urgent needs. The necessary components of this should be the development of new insurance products with differentiation of insurance programs depending on individual choice; improvement of actuarial calculations based on statistical information on predicting the course of the pandemic; periodic improvements in the terms of insurance in the event of adverse events (in particular, an increase in insured amounts and a change in the terms of payments); organizational modernization of support and settlement of insurance claims etc. A huge role in these processes should be assigned to national authorities that perform the functions of the financial market regulator.

Another key promising direction for reformatting national health financing systems should be the introduction of innovative financial mechanisms that can create a foundation for a sufficient level of resource provision of the health care sector in the face of modern challenges by overcoming a number of chronic problematic issues inherent in the health sector, especially in developing countries.

Among such mechanisms, the most optimal and common in foreign practice is a concession. The economic meaning of the concession is that concession agreements are concluded between public authorities and private investors, according to which medical facilities are temporarily, but for a long time (usually from 10 to 50 years), transferred under the management of business entities. In other words, a private investor, in exchange for the remuneration specified in the agreement, receives the rights related to the use and economic management of the resource provision of the medical service facility on the basis of a concession agreement, which, in accordance with legislative norms, has a long term. An important aspect is the fact that when concluding concession agreements, the object of the contract remains in the ownership of the state (Figure 2).

As Yu.V. Shevchuk notes, the use of various forms of public-private partnership in the field of healthcare is due to the fact that the overall goal of the participants' activities can be both commercial and non-commercial, not related to making a profit. The latter corresponds to the profile of enterprises united with state or municipal health care institutions, which are often non-profit organizations [13].

The main advantage of concession agreements is that they can significantly improve the quality of medical services and achieve a high level of efficiency of budget financing. Concession legal relations are a mutually beneficial and fairly widespread form of partnership in world practice. At the same time, the effective implementation of the mechanisms of concession agreements is possible only if the regulatory documents governing legal relations in the field of public-private partnership are coordinate.

We believe that in modern conditions concession agreements should be used to provide healthcare institutions with medical information registration systems, research equipment, systems for monitoring the condition of patients with coronavirus disease, biological safety equipment, as well as in issues of the reconstruction, modernization and re-equipment of medical institutions and facilities, and in pharmaceutical developments in the field of combating the spread of coronavirus disease and the like.

In particular, the conclusion of concession agreements should create the foundation for supporting specialized and highly specialized branches in medicine, the implementation of recovery and rehabilitation in the provision of spa services to patients who have recovered from coronavirus disease, stimulate relevant research and education (in the preparation of management personnel for the medical field).

CONCLUSIONS

Summarizing the above, the following should be noted. Against the backdrop of the global pandemic, national health financing systems in the vast majority of the world's countries have experienced unprecedented pressures and problems both in terms of health insurance due to the huge losses of the insurance industry in 2020, and in terms of budget financing due to limited budgetary resources and the need for government measures to mitigating the negative consequences of falling GDP. In order to improve health care financing systems, it is necessary, on the one hand, to update the forms, methods and tools of the insurance market functioning in terms of the health insurance segment, and on the other hand, it is necessary to introduce new mechanisms for financing the medical sector in the process of combating the spread of coronavirus infection, among which concession agreements occupy an important place.

The coronavirus pandemic has shaken all countries of the world, but at the same time, the fight against its consequences can give a powerful impetus for a radical improvement in the health care sector due primarily to the investment component, rationalization of budget expenditures, building the foundation for technological equipment and the stable development of national health systems responding to new challenges, expanding the list of alternatives for the patient's choice of medical services and organizations, which in the end should become a guarantee of high quality and accessibility of medical care.

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REVIEW ARTICLE

MODERN ASPECTS OF INTRAUTERINE INFECTION

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ABSTRACT

The aim: To analyze the main types of intrauterine infections(IUI), their routes of transmission and features of the clinical picture, as well as the influence of pathogens on the course of pregnancy.

Materials and methods: Data review of native and foreign literature published over the past 5 years.

Conclusions: Summing up, it should be noted the role of infections in the pathogenesis of pathological conditions that form in the perinatal period. This is evidenced by numerous works on the problem of CBS. The most significant perinatal risk factors for fetal infection have been identified. It is proved that in the development of infectious lesions of the fetus, the severity of the disease, the localization of the pathological process, the rate of implementation, and manifestations of the pathology are an important type of pathogen, the path of penetration of microorganisms from mother to fetus to the immune response. Unfortunately, today the problem of preventing CBS is still far from being solved. However, knowledge of the pathogenesis, quality diagnostic methods, effective prevention, and treatment measures can significantly reduce the frequency of VUI and the severity of their consequences for the child.

KEY WORDS: congenital infections, pregnancy outcomes, newborn

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INTRODUCTION

Infectious pathology remains one of the most important and urgent issues of perinatology due to the high mortality and morbidity of the middle of newborns. But before delving into this issue, you need to understand the terms "infection" and "infectious process", as well as intrauterine infection and "congenital infection".

THE AIM

To analyze the main types of intrauterine infections(IUI), their routes of transmission and features of the clinical picture, as well as the influence of pathogens on the course of pregnancy.

MATERIALS AND METHODS

Data review of native and foreign literature published over the past 5 years.

REVIEW AND DISCUSSION

"Infection" is the entry of a microorganism into a macroorganism. This hit did not mean those that allowed detecting the reproduction of the microorganism with the subsequent development of the pathological process. Whereas "infectious process" or "infection" is this dynamic process that is revealed in a macroorganism as a result of penetration into it a microorganism. Therefore, these concepts are not evaluations. Practitioners need to remember that the term "infection" is more epidemic, and "infectious process" is a cyno-epidemiological term.

Another issue that needs to be addressed is the difference between an intrauterine and a congenital infection, which is also often identified in the publication. Intrauterine infection is an infection of the disease for which any infectious fetus occurs in the ante-chi international period. And congenital infection is manifested in utero.

Clinical symptoms of congenital infections, regardless of etiology, are most often intrauterine growth retardation, jaundice, hepatosplenomegaly, exanthema, impaired blood function, and organ. Inclusion in the new technology of combining with certain symptoms the required results of the analysis, assessment, the presence of certain characteristics of diseases that do not allow to decipher clinically. In such cases, you can use the term "TORCH" - syndrome.

What is the history of this syndrome? In 1971, Andre Namias, from a large number of diseases, isolated viral, bacterial and other infections, which with a wide variety of structural and biological properties of the pathogen cause similar clinical manifestations in children. systems.

Under the term "TORCH" - infections refer to congenital infectious diseases, the etiology of which still remains undeciphered. The abbreviation of this term consists of the first letters of the diseases that most often cause intrauterine infection.

T (toxoplasmosis) - Toxoplasmosis
O (Other diseases) - other diseases
R (rubella) - rubella
C (Cytomegalovirus) - cytomegalovirus infection
H (herpes simplex virus) is a herpes simplex virus

According to statistics from the Ministry of Health, infant mortality due to generalized intrauterine infections is 18-20%, about 70 out of 350 cases per year. Published statistics show that 27 to 36% of live births, including more than 2/3 of premature babies, are infected in utero. Thus, it is proved that in the structure of infant mortality infectious pathology is up to 65-70%, ie is one of the leading causes of death in children in the first month of life. The high mortality rate of newborns determines the importance of timely diagnosis and adequate treatment of perinatal infections. Among the surviving children, disability occurs in almost 50% of cases. The share of mortality from congenital malformations is 30-35%, 1850 infants die before the age of one year, and in general, in Ukraine, more than 35,000 registered children with congenital malformations. [6] The severity of the infectious process in the mother and fetus does not always correlate with each other. Mild, asymptomatic, or asymptomatic infection of the mother, caused by various infectious agents, may be accompanied by severe damage to organs and systems of the fetus or its death. At the same time, acute and severe infection in the mother is not necessarily fatal to the fetus. Difficulties in ante- and postnatal diagnosis of intrauterine infection (IUI) are associated with the widespread prevalence of persistent infections and opportunistic agents in the human population, the ambiguity of the possible implementation of the infectious process, and non-specific manifestations. [7,] The process in the study of fetal infection (FI)is associated with the development and widespread implementation in health care practice of fundamentally new diagnostic technologies - highly sensitive and specific methods of enzyme-linked immunosorbent assay and genodiagnostics. In this regard, the assessment of the effectiveness of modern specific diagnostics of FI becomes especially relevant. [8] Invasive methods of prenatal diagnosis (cordocentesis, amniocentesis, chorionic villus sampling) for the detection of FI markers are rarely used due to a large number of contraindications and the possibility of pregnancy complications. Indirect methods are more often used: detection of the pathogen, its nucleic acids, and antigens in clinical material in pregnant women, specific antibodies in serum. Of particular interest in improving the prenatal diagnosis of FI is the determination of the diagnostic and prognostic significance of marker-producing herpesvirus (HSV) and cytomegalovirus (CMV) infection. After primary infection, viruses of the Herpesviridae family in the form of a nucleotide can be present for life in the cell of nerve ganglia, secretory glands, lymphoreticular cells of the kidneys, lymphoblasts of B-lymphocytes. With primary infection and reactivation of latent infection, the virus actively reproduces and is excreted, specific class M immunoglobulins appear, and low-avidity antibodies to premature proteins are markers of productive infection. Activation of the infection in pregnant women can be both clinical and asymptomatic, and the virus can enter the placenta and cause destructive processes. The risk of infection and fetal disease in the detection of markers of productive herpes infection in pregnant women has not been studied. [9] Determining the role of asymptomatic, persistent, chronic, and recurrent maternal infections in the formation of perinatal losses and congenital malformations remains an unresolved and controversial issue. Viruses have unique properties that contribute to the development of intrauterine infection, damage to embryonic and fetal cells. The key pathogenetic mechanism of viral infection is the incorporation of foreign genetic material into the cell. An important component of the pathogenetic action of the virus on the cells of the embryo or fetus is a violation of the mitosis of infected cells, their cytolysis, chromosomal aberrations, ie direct cytopathic, teratogenic, and mutagenic effects. Indirect exposure to viruses is associated with the development of placentitis, endometritis, pathology of the amniotic membranes.

New perspectives in the study of the prevalence, pathogenesis, etiology of FI opens the use of a highly sensitive molecular biological method of PCR, based on the detection of a specific nucleotide sequence inherent in the DNA or RNA of the agent. The method allows assessing the role of FI in the development of perinatal losses, congenital anomalies at a qualitatively new level.

Morphofunctional condition of the placenta in various pathological conditions, including infection, can affect the prognosis of the fetus in different directions. First, it is a decrease in energy, respiratory and other biochemical processes, violation of the hormonal status of the placenta, which is reflected in the levels of hormones of the mother and fetus, changes in tolerant immunological relations in the system "mother - fetus". Secondly, it is a lesion that facilitates the entry of an infectious agent into the fetus and the development of pathological changes in its organs. They are closely intertwined, creating a cascade of pathological reactions that cause the existence of the fetus in extreme conditions, and in some cases - and before his death. [9-11]

Ways of intrauterine infection

- Ascending - from the ancestral ways. Intensive reproduction of microorganisms in the vagina and cervical canal, including opportunistic pathogens, leads to their penetration into the uterine cavity. Next, they colonize the basal layer of the decidual membrane. From here the pathogen can get into blood vessels of a fruit and further cause a choriovasculitis, or, more often - in an amnion cavity with the development of bacterial amnionitis. Clinical and experimental microbiological studies have shown that amniotic fluid is the main pathogenetic link in the mechanism of infection of the fetus.

Women's amniotic fluid has antimicrobial activity, however, it is not expressed concerning clinical strains of opportunistic pathogens, which is manifested by delayed rather than inhibited growth of flora. The bacteriostatic effect is short-lived, and amniotic fluid becomes an environment for the accumulation of opportunistic pathogens.

The fetus is in an infected environment, and infection can occur by swallowing, during intrauterine respiratory movements, or aspiration of infected amniotic fluid during childbirth. The ascending path of infection is more often caused by opportunistic bacteria (Escherichia coli, enterococci, etc.), as well as mycoplasmas, chlamydia, fungi of the genus Candida and only certain viruses, in particular - herpes simplex. [9]

- Hematogenous (transplacental) - from foci of infection located in the mother's body extragenital or in the myometrium (all congenital viral infections, as well as syphilis, toxoplasmosis).

The presence of the pathogen in the mother's blood may be accompanied by certain symptoms or signs (Influenza, pyelonephritis, etc.), but can be asymptomatic or manifest itself in the form of nonspecific signs (rash, lymphadenopathy, etc.). The pathogen, breaking the placental barrier, enters the bloodstream of the fetus. Often there is a generalized lesion of the fetus - intrauterine sepsis.

Hematogenous infection is more characteristic of viruses, mycoplasmas, chlamydia, treponemes, listeria, toxoplasma, Mycobacterium tuberculosis. This route of infection has a characteristic pathomorphological picture of inflammatory changes in the manure and fetal organs.

- Transdecidal (transmural) - the source of infection is under the endometrium. this route of infection of the fetus is more often associated with purulent-inflammatory diseases of the mother's genitals in the past. Infection of the fetus can threaten endometritis and the presence of contraceptives in the uterus.

- Descending - through the fallopian tubes. The descending route of infection begins with chronic foci of inflammation in the ovaries and fallopian tubes due to gonorrhea, mycoplasma, and chlamydial infections. The descending way of defeat is often observed in patients with acute pathology of abdominal organs. A classic example is an acute appendicitis.

- Iatrogenic route of infection. Currently, there is another way of infection, which can occur when taking chorionic villi for examination, especially if it is performed transcervical during amniocentesis, fetoscopy, fence for examination of fetal blood, an intrauterine blood transfusion.

- The contact path is realized during childbirth more often when the fetus passes through the infected birth canal.

- Mixed. In this case, there are two or more possible ways of infection.

CLINICAL SYNDROMES OF INTRAUTERINE INFECTION

Unfortunately, many IUIs in the neonatal period do not have a specific clinical picture. Indications for examination at IUI are nonspecific symptoms of the infectious process. In newborns, the clinical manifestations of the infectious process may be:

• general symptoms - loss of appetite, large initial weight loss (10% or more) and its slow recovery, repeated weight loss, flat weight curve, malnutrition, lethargy, sclera, pale skin with a grayish tinge, jaundice, hemorrhagic syndrome, edema ;

• respiratory disorders - tachypnea or shortness of breath, apnea, cyanosis, the participation of accessory muscles in the act of breathing;

• gastrointestinal symptoms - belching, vomiting, enlarged abdomen, diarrhea, the plasticity of the anterior abdominal wall, hepatosplenomegaly;

• cardiovascular disorders - tachycardia, muffled heart sounds, dilated heart, pale and marbled skin, cold extremities, decreased subcutaneous tissue turgor, edema, pasty, hypotension;

• signs of CNS damage - decreased muscle tone and reflexes, as well as motor activity and sucking reflex, convulsions, hyperexcitability;

• hematological abnormalities - anemia, thrombocytopenia, changes in white blood cell count, hypocoagulation, etc.

Signs of the disease due to intrauterine infection of the fetus appear during the first 3 days of life. It should be borne in mind that intranatal infection may have a longer incubation period of the disease. Great difficulties in the differential diagnosis arise due to the similarity of the symptoms of infectious toxicosis with the syndromes of maladaptation caused by perinatal hypoxia, especially in premature infants. [13,14]

It should be noted that a comprehensive examination of women to detect infection with pathogens of prenatal infections, especially the TORCH group, should be performed before pregnancy. Treatment of these infections is one of the main tasks of pre-pregnancy training. However, most of these infections are not treated effectively or at all. All women of childbearing potential planning a pregnancy should be tested for antibodies to the rubella virus. Seronegative women are recommended to be vaccinated, followed by contraception for three months. It is also advisable to vaccinate against hepatitis B before pregnancy. This is the only and best method of preventing intrauterine infection during pregnancy to date. [15-17]

TOXOPLASMOSIS

Toxoplasmosis - protozoonosis, characterized by a variety of variants and polymorphism of clinical manifestations, is currently primarily considered as an opportunistic infection and is an urgent medical and social problem.

Toxoplasmosis invasion is widespread, almost worldwide. Affected populations in different countries depend on sanitary and hygienic conditions, the peculiarities of the population's diet, the influence of environmental factors, and the frequency of immunodeficiency conditions.

The causative agent of toxoplasmosis is the obligate intracellular parasite Toxoplasma gondii. The name of the genus Toxoplasma (Greek Toxon - arc, plasma - decorated) is determined by the shape of the parasite in the form of a crescent or a slice of orange at the stage of rapid reproduction in the cells of the intermediate host. Toxoplasma - a representative of the type Sporophytes, class Coccidia. It was first described

in 1909 by S. Nicollen and L. Manso, who discovered it in Tunisia in rodents (Ctenodactylus gundi).

The sexual cycle of toxoplasma development occurs in the intestinal epithelium of members of the feline family, including domestic cats. Excreted with the feces of animals, parasites in the form of oocysts for a long time retain their viability in the environment (1.5-2 years). The asexual cycle of toxoplasma development is realized in the human body or various mammals.

The main routes of transmission include: 1) food; 2) contact; 3) transplacental; 4) parenteral.

The vast majority of people are resistant to toxoplasmosis, as the human body with full immunity easily copes with toxoplasmosis. Thus, in adults, screening antibodies to Toxoplasma are detected in 40-90% of cases. Toxoplasma elicits an intense cell-mediated immune response.

Toxoplasmosis infestation in humans is found everywhere, on all continents, and in all climatic zones. The prevalence of toxoplasmosis is higher in areas with hot and humid climates, as well as among the rural population.

There are natural and synanthropic foci of acquired toxoplasmosis. In natural foci, the circulation of the pathogen occurs mainly in the chain of prey - a predator, in which the final host is a member of the feline family, and the intermediate - numerous mammals, animals, and birds. In natural foci, people are rarely infected. In synanthropic foci, the pathogen of toxoplasmosis circulates with the participation of cats as the final hosts in which the parasite reproduces sexually. Among the intermediate hosts, the most affected are pigs, sheep, rabbits, chickens, mice, and wild birds - sparrows. Domestic mammals and synanthropic birds are infected with Toxoplasma much more often than wild ones. Man for Toxoplasma is an intermediate host; in its cells, Toxoplasma reproduces asexually.

The main sources of toxoplasmosis infection in humans are pets. Of the domestic carnivores, the leading role is played by the cat, which spreads toxoplasma oocysts in the environment. An important epidemiological role in human infection with toxoplasmosis is played by farm animals, whose meat may contain toxoplasmosis cysts, as well as the selection of sick animals at birth.

Women are usually infected slightly more often than men, due to the habit of many of them tasting raw minced meat.

In an organism with good immunoresistance, toxoplasmosis rarely gives typical manifest forms: in 95-99% this disease is asymptomatic and remains undiagnosed due to the absence of pathognomonic signs [8, 34].

Clinical manifestations are multifaceted and depend on the initial state of the body's immune system. According to the classification of Siim (Siim, 1971), taking into account the predominant organ pathology, there are 5 clinical forms: lymphonodular, generalized (exanthemous), myocardial, encephalitic, ocular. Other classifications also distinguish between intestinal (abdominal) and pulmonary forms of acute toxoplasmosis.

There are alternative classifications of toxoplasmosis by the method of infection: 1) acquired toxoplasmosis, 2) congenital toxoplasmosis; by clinical manifestations: 1) primary latent form; 2) acute toxoplasmosis; 3) primary-chronic form (expressed and erased); 4) secondary-chronic form (expressed and erased); 5) secondary-latent form (with or without residual phenomena).

The incubation period lasts from 3 to 21 days but can last up to several months.[35-38]

The duration of the incubation period depends on the virulence of Toxoplasma, the mass of infection, and the state of the premorbid background (the presence of congenital or acquired immunodeficiency and its severity).

Acute toxoplasmosis, which developed as a result of primary infection, begins acutely and proceeds with severe intoxication, fever, and CNS lesions such as meningitis. Subjectively, the patient will be disturbed by chills with fever, arthralgia, myalgia, cardialgia, impaired vision, itchy skin, sleep disturbances, headaches. objectively, you can detect micropolyadenitis, hepatosplenomegaly, hyperhidrosis, ecchymoses, petechiae, chorioretinitis, iridocyclitis.

In the presence of pregnancy, toxoplasmosis causes its termination at an early stage, stillbirth, birth of children with developmental abnormalities, and damage to the CNS and other organs.

Approximately 5-7% of women become infected for the first time during pregnancy. There is a consensus on the threatening effects of acute toxoplasmosis on pregnancy. When a woman is infected in the third trimester of pregnancy, latent or primary chronic toxoplasmosis is most often formed, which does not endanger the life of the child and does not cause a delay in neuropsychological development in children. [18, 19-24]

RUBELLA

Rubella - a highly contagious anthroponotic viral infection, almost harmless to adults, but extremely dangerous for women of childbearing age, due to the high probability of intrauterine infection of the fetus during pregnancy. The causative agent of this infection is the rubella virus, which belongs to the family Togaviride genus Rubivirus. The incubation period of the disease can last from 16 to 21 days, the main symptoms are enlargement and soreness of the lymph nodes and spotted or maculopapular rash, which begins on the patient's face, then passes to the body and limbs.

In children, the disease is usually mild, with symptoms including rash, fever (<39 ° C), vomiting, and mild conjunctivitis. The rash, which appears in 50-80% of cases, usually first appears on the face and neck, then falls below the body and lasts 1-3 days. Swollen lymph nodes behind the ears and on the neck are the most characteristic clinical sign. Infected adults, more often in women, may develop arthritis with joint pain, which usually lasts 3-10 days.

After infection, the virus spreads in the human body for 5-7 days. Symptoms usually appear 2-3 weeks after exposure. The most infectious period usually occurs 1-5 days after the rash.

If a woman is infected with the rubella virus in early pregnancy, the probability that she will transmit the virus to the fetus is 90%. This can cause fetal death or congenital

rubella syndrome (CNS). Infants with IBD can shed the virus for a year or more after birth. Babies with IBD can shed the virus for a year or more after birth. Children with IBD may suffer from hearing impairments, eye defects, heart defects, and other lifelong forms of disability, including autism, diabetes, and thyroid dysfunction. Many of these disorders require expensive therapy, surgery, and other expensive medical care.

The highest risk of SVC exists in countries where women of childbearing age are not immune to the disease (which is produced either as a result of vaccination or after previous rubella). Before the introduction of the vaccine, up to 4 children per 1,000 live births were born with SVC. [18, 25-27]

CYTOMEGALOVIRUS INFECTION

Diseases caused by cytomegalovirus (CMV) are anthroponotic viral infections and differ in a variety of clinical manifestations: from latent to generalized forms with damage to the nervous system and internal organs. The essence of the problem of CMVI is that it refers to the so-called opportunistic infections, the clinical manifestation of which is possible only in conditions of primary or secondary immunodeficiency. In people with a normally functioning immune system in the vast majority of cases, the infection is of the type of virus. On average, 90-95% of the adult population show antibodies to CMV [1].

The number of seropositive patients in different countries ranges on average from 44 to 85%, with patients from 0.2 to 3%. The prevalence of cytomegalovirus infection, the possibility of long-term persistence of the pathogen in the human body with damage to various organs and systems, the difficulty of laboratory diagnosis, lack of reliable therapeutic and prophylactic agents convincingly emphasize the relevance of this infection.

The causative agent of cytomegalovirus infection is cytomegalovirus (Cytomegalovirus hominis) - an opportunistic pathogen belonging to the family of beta-herpesviruses of the fifth type. The human CMV genome is the largest of all the genomes of the herpesvirus family. The source of infection is a sick person with one or another form of the disease or a chronic virus carrier, which is most dangerous in the active phase of the primary infection or during the exacerbation of the infection.

The ways of CMVI transmission are vertical, sexual, parenteral, aspiration, oral. Transmission factors are blood, cervical and vaginal secretions, semen, breast milk. Infection can also occur through donor organs and tissues transplanted to recipients. CMV is stored for a long time in leukocytes, which leads to the risk of developing this infection in recipients of blood and its components.

Primary infection of immunocompetent adults usually occurs asymptomatically and only in 5% of cases in the form of the mononucleosis-like syndrome, characterized by fever, asthenia, in the blood - lymphomonocytosis, atypical mononuclear cells, although not always characteristic of angina and lymphadenopathy.

Hepatomegaly is observed in 100% of cases, is described as an initial manifestation of CMVI or as a concomitant mononucleosis-129-like syndrome of granulomatous hepatitis. In the absence of pathology of the immune system, acute CMVI becomes latent with the lifelong presence of the virus in the human body. Detection of specific IgM in people with normal immunity indicates that they have an active infection and provides an earlier diagnosis of CMVI.

Seroconversion is a reliable sign of primary CMVI. A high titer of CMV IgM indicates a primary infection because recurrence of CMVI rarely gives high titers of IgM. However, not all individuals can produce IgM antibodies.

In immunocompromised individuals, IgM to CMV does not form even in the case of clinically severe infection. Determination of anti-CMV IgG in the dynamics by ELISA with a fourfold increase in antibody titers with confidence indicates an acute infectious process. [18, 28,29]

HERPES SIMPLEX VIRUS

According to the WHO, this is currently a pandemic: from 60% to 90% of adults and children in the world are infected with herpes viruses. In 2016, an estimated 3.7 billion people under the age of 50, or 67% of the population, had HSV-1 infection (oral or genital). Estimated prevalence of the infection was highest in Africa (88%) and lowest in the Americas (45%).

With respect to genital HSV-1 infection, between 122 million to 192 million people aged 15-49-years were estimated to have genital HSV-1 infection worldwide in 2016, but prevalence varied substantially by region. Most genital HSV-1 infections are estimated to occur in the Americas, Europe and Western Pacific, where HSV-1 continues to be acquired well into adulthood.[30-39]

Genital herpes (GH), a common cause of HSV infection, is one of the most common sexually transmitted diseases and differs from other diseases in this group by a lifelong carrier of the pathogen in the human body, which determines a high percentage of recurrent forms of the disease.

The prevalence of GH in the population is judged by the frequency of detection of antibodies to this serotype of the virus. Antibodies to HSV-2 are found in all population groups.

The frequency of detection of GG is determined by the studied citizens and socio-economic conditions, it increases with age and correlates with the number of sexual partners.

Seroepidemiological studies have shown a significant difference between the prevalence of seropositivity and the actual incidence of GH, which in Western Europe exceeds 80 cases per 100 thousand population, and in the United States is approaching 200 cases per 100 thousand inhabitants. According to US researchers, about 30 million adults in the United States suffer from recurrent genital herpes (RGH). And every year about 500 thousand new cases are registered. It is estimated that in developed countries, 10-20% of the adult population may suffer.

Herpes infection, along with cytomegalovirus is one of the main factors of the fetus and newborn, causes an increase in the number of miscarriages, premature births,

the birth of children with the pathology of the CNS and internal organs. Infection of the child occurs during vertical, hematogenous, transplacental, as well as intra- and postnatal transmission. This is especially common in the presence of active manifestations of herpes on the skin and mucous membranes in the mother.

Usually, in GH the infectious agent is HSV-2, but in 10-6% of cases, the cause of the disease may be HSV-1, which is due to household and oral-genital routes of infection. The entrance gates are the skin and mucous membranes of the external genitalia and vagina.

At primary infection, the virus rises from the site of introduction through the peripheral nerves to the spinal and cerebral ganglia and sometimes reaches them due to viremia. Here he remains "asleep" and often invulnerable to anti-virus attacks. Upon reactivation, the GH virus migrates for a long time along the peripheral nerves, causing irritation of the nerve endings and very characteristic and unpleasant sensations of itching and burning. These phenomena usually precede the appearance of the vesicular rash.

Even with high levels of circulating virus-neutralizing antibodies, recurrences of herpes infection are possible, as the herpes virus spreads inside the nervous tissue, passing from one cell to another, avoiding contact with antibodies. Thus, functioning virus-neutralizing antibodies do not prevent a recurrence, although they prevent the spread of infection. According to IS Markov (2001), HSV has a "strange pantropism." It is known for its high tropism to tissues of ectodermal origin, in connection with which the most common lesions of the skin, mucous membranes, central and peripheral nervous systems. Lesions of vital internal organs, primarily the liver, are caused by the tropism of the virus also to tissues of endodermal origin.

Such almost universal tropism has led to a significant polymorphism of clinical manifestations, in connection with which patients often come into the field of view of doctors of different specialties.

Even though the mechanism of recurrence of herpes infection is not completely clear, clinically significant are many factors and their combinations that cause exacerbation of latent viral infection: premenstrual and menstrual periods, fatigue, stress (emotional and physiological imbalance), excessive ultrasound. sun, drafts, excessive cooling, immunodeficiency states of both genital and extragenital genesis, sexual contact or other irritating mechanical or chemical exposure to the external genitalia, intercurrent infection, etc.

The most realistic option to join the host of GG owners is direct contact with secretions from an infected patient. And it is not necessary that he currently has any painful symptoms.

The incubation period of primary HH varies from 2 to 12 days (according to some data from 1 to 26 days), on average 6-7 days. A typical picture of the manifestation of GG - the appearance on the mucous membranes of the genitals and adjacent areas of the skin of single or multiple vesicular (vesicular) elements that occur on an erythematous background. After 1-2 days, these bubbles open, forming moist painful erosions, rarely ulcers, heal under the crust or without it. In women, there is often the so-called acute edematous vulvovaginitis (Boralevi F., Geniaux M., 1996). Usually, the primary attack of GG is quite difficult - pronounced general intoxication: fever, weakness, headache and muscle pain, dysuric phenomena. Often in primary infection, there is multiple localization of lesions, as well as enlargement and soreness of the inguinal lymph nodes.

The period of predictors (prodromal phase) is observed usually at recurrent GG, meets at half of the patients, and lasts about 24 h (with considerable variability in a clinical course). If the prodromal period is diagnosed in time, it can allow early treatment, which is more likely to be effective.

The location of lesions on the genitals is determined by the entrance gate of infection. In men, the manifestations of GG are usually localized on the foreskin, head, and body of the penis, as well as the perianal. The female genitalia is affected in the area of the labia, clitoris, perineum, vagina, and anus. It is also possible lesions of the cervix in the form of diffuse inflammation with erosion, the formation of largely isolated ulcers, sometimes even with the phenomena of necrosis.

The acute period of primary HH can reach 3-5 weeks, but sometimes the infection is latent, immediately passing into a latent phase.

Against the background of erythema, the blisters are ulcerated, covered with crusts, cuttings usually without scars. About a quarter of patients have neuralgia. Positive dynamics in the development of local manifestations are less pronounced in wet areas of the genitals. Erosions and ulcers localized in these areas heal much longer than on dry skin.

Severe pain and tissue destruction can cause urinary retention (usually in the initial attack). More rare complications in the acute stage are herpetic eczema, parasitism, proctitis, bilateral interstitial pneumonia, hepatitis, polymorphic erythema, aseptic meningitis, myelitis, etc. [18,30-32]

CONCLUSIONS

Summing up, it should be noted the role of infections in the pathogenesis of pathological conditions that form in the perinatal period. This is evidenced by numerous works on the problem of CBS. The most significant perinatal risk factors for fetal infection have been identified. It is proved that in the development of infectious lesions of the fetus, the severity of the disease, the localization of the pathological process, the rate of implementation, and manifestations of the pathology are an important type of pathogen, the path of penetration of microorganisms from mother to fetus to the immune response. Unfortunately, today the problem of preventing CBS is still far from being solved. However, knowledge of the pathogenesis, quality diagnostic methods, effective prevention, and treatment measures can significantly reduce the frequency of VUI and the severity of their consequences for the child.

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REVIEW ARTICLE

FIRST BILATERAL LUNG TRANSPLANTATION VIA ANTEROLATERAL THORACOTOMY IN UKRAINE

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ABSTRACT

The aim: The aim of this work is to describe the clinical case of the first lung transplant in Ukraine through anterolateral access and to analyze management of patient in the postoperative period, followed by assessment of his general condition and quality of life.

Materials and methods: Analysis of the clinical case of the patient who underwent the first lung transplantation in Ukraine, including a description of surgery, anesthesia, and postoperative recovery.

Conclusions: Thus, based on the data of the world literature, as well as on the results of treatment on the basis of Municipal Non-Profit Enterprise " Lviv Emergency Clinical Hospital" we can conclude that lung transplantation is one of the most effective methods of treatment of patients with end-stage lung disease. This operation improves patients' quality of life, general well-being and ability to physical activity.

KEY WORDS: organ transplantation, immunosuppression, quality of life

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INTRODUCTION

Lung transplantation is a common method of treatment of end-stage lung diseases all over the world. In many countries, such treatment is a 'golden standard' for patients with chronic lung diseases [1].

The first human lung transplant was performed in 1963. The patient undergoing the transplant had been diagnosed with advanced lung cancer and a lung abscess. Unfortunately, on the 18th day after transplantation, the recipient died, due to the development of renal failure [2]. However, this case demonstrated that lung transplantation was technically possible and that rejection of the transplanted organs could be avoided with available immunosuppressive agents.

There were several more transplantations performed, for the next 15 years after the first attempt of lung transplantation but most of the recipients died after surgery. Common causes of death were complications associated with bronchial anastomosis. The first successful transplantation of the cardiopulmonary complex was performed in 1981 on a patient with idiopathic pulmonary arterial hypertension. The first successful single lung transplantation was performed in 1983 for idiopathic cystic fibrosis, and soon in 1986, there was the first double lung transplant surgery for emphysema. Due to the improvement of surgical methods, the invention and implementation of cyclosporine successful outcomes after lung transplantation became reality. Over the next few years, lung transplantation became a method of treating patients with irreversible lung changes.

For the past 35 years, lung transplantation became almost the only treatment option for patients with end-stage lung disease, such as chronic obstructive pulmonary disease, pulmonary hypertension, or cystic fibrosis.

Patients selecting criteria for lung transplantation have been developed and improved for a long time. In 2014, the International Society for Heart and Lung Transplantation (ISHLT) published an updated consensus on the selection of candidates for lung transplantation [3]. Due to the consensus lung transplantation should be considered for adults with chronic end-stage lung disease who have all of the following criteria [4]:

high (> 50%) risk of death from lung disease within 2 years if lung transplantation is not performed;

high (> 80%) probability of survival for at least 90 days after lung transplantation;

high (> 80%) probability of 5-year survival after transplantation from a general medical point of view, if the function of the transplanted organ remains adequate.

Although organ transplantation has been developed on a high level, there is a relatively small number of lung transplantations, which is associated with a small number of donor's lungs that are vulnerable to potential complications which often occur before and after the death, such as chest trauma, aspiration, lung damage associated with ventilation, pneumonia, and neurogenic pulmonary edema. This is one of the reasons for such a difference between lungs and other organ removals from dead donors. Only 15 percent of all dead donors can have their lungs removed, compared to the

Table I. Dynamics of laboratory results

Laboratory results	day	day	day
WBC,	27,3	14,9	8
ALT, mmol/l	23,1	30,3	18
AST, mmol/l	68,5	31,2	16
Bilirubin tot., mmol/l	25,3	9,97	3,68
Urea, mmol/l	6,9	23,5	4,4

Table II. Dynamics of results of arterial blood gas testing

Arterial blood gas	day	day	day
pC, mm/Hg	24,9	51,8	39
p, mm/Hg	292	53,6	89
BUN, mg/dl	13	54	31

88 percent of kidneys and liver removal, and 30 percent of heart removal from all dead donors. However, studies show that in some cases perfusion and recovery of the lung ex vivo can reduce lung damage and allow the transplantation of those donor's lungs that were previously considered unsuitable [5].

Nowadays there are lots of researches on assessing the quality of a patient's life after lung transplantation. According to the results of these studies the quality of life after transplantation as well as physical activity increases, general well-being improves, shortness of breath and anxiety are reduced and so people after lung transplantation can return to their common way of life. [6]. More than 80% have no activity restrictions.

Moreover, improvement of life quality after lung transplantation has been documented in numerous publications [7]. A negative impact on the quality of life associated with health can be infections and episodes of acute rejection of the transplant [8]. Even if the benefits of survival are modest in themselves, the prospect of improving quality of life and survival is often a motivation for transplantation for many recipients, and nearly 90 percent of survivors are satisfied with their decision to have a transplant.

THE AIM

The aim of this work is to describe the clinical case of the first lung transplant in Ukraine through anterolateral access and to analyze management of patient in the postoperative period, followed by assessment of his general condition and quality of life.

MATERIALS AND METHODS

Analysis of the clinical case of the patient who underwent the first lung transplantation in Ukraine, including a description of surgery, anesthesia, and postoperative recovery.

REVIEW AND DISCUSSION

On the 21st of September, the first lung transplantation was performed on the base of the Municipal Non-Profit Enterprise " Lviv Emergency Clinical Hospital". Our surgeons were operating with the assistance of colleagues from Silesian Centre for Heart Diseases in Zabrze full professor of medicine Marian Zembala, Marek Ochman, MD, PhD who headed the surgery himself, Maciej Urlik, MD, a specialist in cardiac surgery and clinical transplantology, Tomasz Stącel, MD, PhD, a specialist in cardiac surgery and clinical transplantology, Piotr Pasek, MD, PhD, a specialist in cardiac surgery, Anna Pióro, MD, a specialist in anaestesiology and intensive care, Łukasz Lech, M.Sc., a physiotherapist,

Patient B. was hospitalized to the cardiac surgery and heart transplantation department with complaints of severe shortness of breath while doing minimal exercises, dry cough, intermittent leg swelling, and general weakness.

The first symptoms appeared nearly 16 years ago when he began to notice shortness of breath during normal exercises. His condition gradually deteriorated and approximately 8 years ago he was diagnosed with bronchiectasis. Since that time, patient B. has been on a waiting list for lung transplantation.

During the pre-operative period he underwent several examinations. On the abdominal ultrasound were found the signs of nephrolithiasis. By the results of the chest cavity CT scan, he had emphysema, bilateral lung fibrosis, nodal peribronchial inclusion in S1 of the left lung which could be a peripheral neo process and bronchiectasis. During heart ultrasound were detected chronic pulmonary heart in the stage of compensation, insufficiency of the tricuspid valve of a mild degree, overall contractility of the left ventricle is satisfactory with the EF 65%.

On the 21st of September was performed bilaterally sequential lung transplantation.

Anesthesiological aspects of the surgery. Intraoperative monitoring included: ECG, invasive blood pressure measurement from the left radial and right femoral arteries, Swan-Gantz catheter installed through the right internal jugular vein, Foley catheter, oropharyngeal temperature sensor, external self-adhesive defibrillator electrodes, nasogastric tube, and cerebral oximetry.

Venous access was provided by catheterization of two peripheral veins and two central veins (right internal

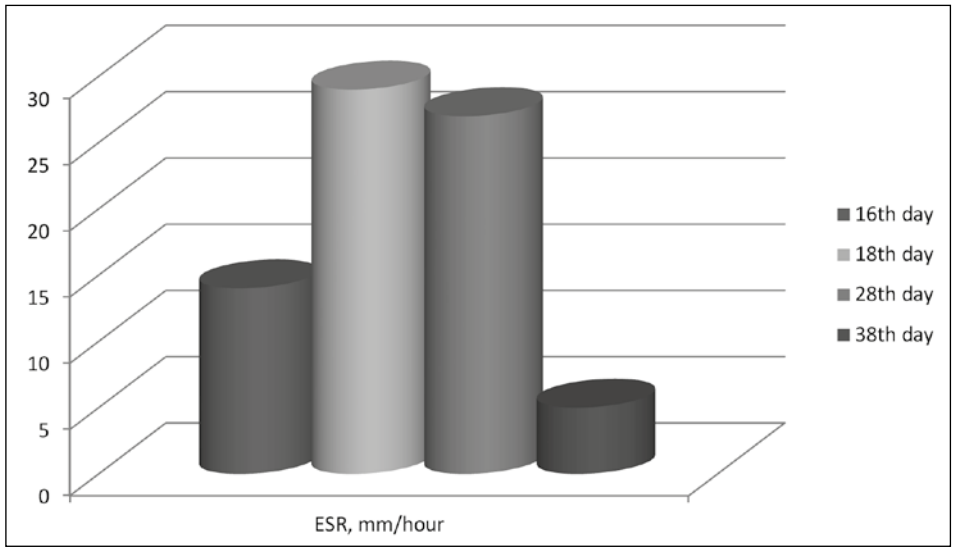

Fig. 1. Dynamics of the ESR

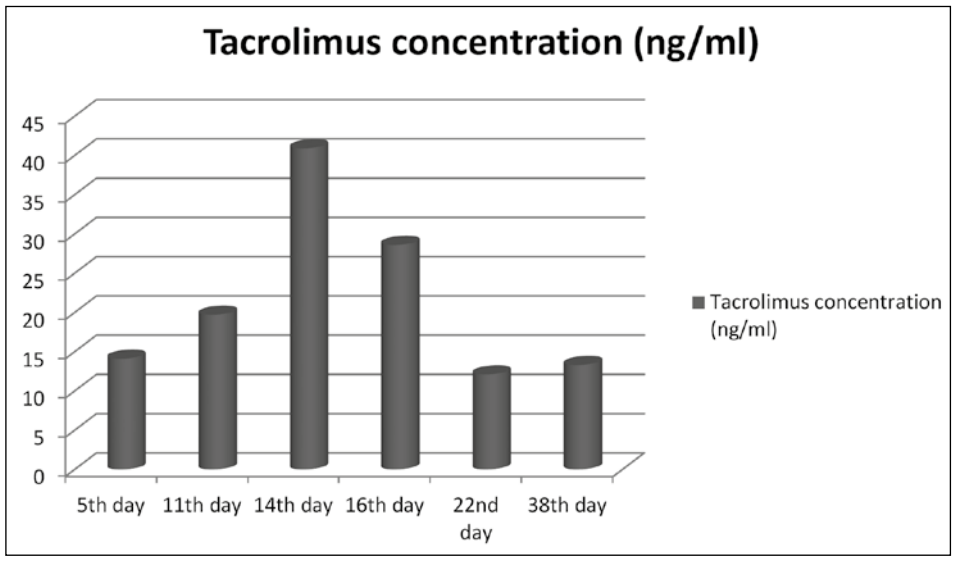

Fig. 2. Dynamics of tacrolimus concentration

jugular and right femoral veins) with the installation of 4-lumen catheters.

Induction. Propofol 200 mg; fentanyl 0.2 mg; atracurium 70 mg.

Intubation was performed using a separate double luminal left-sided endobronchial tube size 41Fr.

Anesthesiological management was performed by total intravenous anesthesia, supported by the continuous infusion of propofol, fentanyl, atracurium using syringe pumps. Mechanical ventilation was performed with pressure control and with FiO2 100%.

Infusion therapy during surgery included colloids, albumin 10%, red blood cells and autoreinfusion (using a cell saver). Medication during the surgery included antibiotics (meropenem 2 g for 2 h by continuous infusion with a syringe pump every 8 h; linezolid 600 mg every 12 h IV infusion); antifungal drugs (voriconazole 200 mg every 12 hours) and immunosuppressants (methylprednisolone 1 g IV before induction; tacrolimus 0.5 mg/50 ml by continuous infusion with a syringe pump before extubation).

Hemodynamic support was provided by prolonged infusion of norepinephrine. Arterial blood gas monitoring and correction if needed was performed every hour as well as electrolytes and blood glucose levels.

Lung transplantation consisted of 2 stages. First one: donor lungs exclusion after heart extraction. Lung protection was administered by infusion of Lung Protect fluid through catheterized lung artery with 16Fr catheter. Then lungs were separated from the soft tissues. The trachea was sutured with a linear suturing device 5 rings above the carina and cut off.

Second stage: lung transplantation itself. The preparation of lungs included the division of the pulmonary complex into right and left lungs. Anterolateral thoracotomy was performed. During right-sided pneumonectomy the ventilation was provided by single left lung ventilation. Bronchial anastomosis, arterial anastomosis, anastomosis of the left atrium were made. The clamp from the lung artery and vein was removed. After that deaeration and reperfusion were conducted, right lung transplantation is performed similarly while mechanical ventilation was performed on the transplanted lung. Pleural cavities were drained. The wound was sutured and on the final stage diagnostic bronchoscopy was performed on the operating table.

After surgery patient B. was hospitalized to the ICU at 08:10 p.m. on controlled breathing through the endotracheal tube. T 36.6 °C, BP 110/60 mm Hg, HR 82/min, SpO2 99%. Ventilation (Vt- 8 ml/kg, PEEP - 8 with a gradual reduction to 4, bpm 12-18 depending on pCO2, FiO2 - 100% with a gradual reduction of up to 30%). Inhalation of NO was stopped 1 h before extubation. There was a constant infusion of norepinephrine, tacrolimus. The drainage system was located in the left and right pleural cavities, from which the hemorrhagic contents were allocated.

In the early postoperative period, arterial blood gas measurements, electrolyte levels, and level of glycemia were monitored and corrected every 2-4 hours, biochemical examinations, blood coagulation test, CRP, PCT, tacrolimus concentration, and chest X-Ray daily.

At 04:15 a.m., after the restoration of adequate spontaneous breathing, satisfactory muscle tone, and consciousness, the patient was extubated. The insufflation of oxygen through the mask was adjusted with a flow rate of 4 l/min. On the 22nd of September patient was adjusted with non-invasive mechanical ventilation due to the increase of pCO2 according to the arterial blood gases (pH 7.21, pCO2 65 mmHg, pO2 163.8 mmHg). When arterial blood gas measurement became normal (pH 7.31, pCO2 49.7 mmHg, pO2 86.1 mmHg), non-invasive ventilation was stopped, however, the administration of the norepinephrine continued.

On the 23rd of September both upper drainages were removed. Lower drainages were in a state of active aspiration.

Activation (first getting out of bed) 48 hours after transfer to ICU.

On the 24th of September to treat atrial fibrillation and restore sinus rhythm, electrical cardioversion with a charge of 150 kJ was performed, the rhythm was restored for the first time. Both lower pleural drainages were removed. X-ray examination revealed pneumothorax on the right and so right pleural cavity was drained and put on active aspiration.

On September 25th arterial and urinary catheters and on the 26 of September the central venous catheter were removed. The results of histological lung examination showed chronic obstructive bronchitis, peribronchial sclerosis, focal interstitial pneumosclerosis and bilateral subpleural bullous emphysema of the lungs, focal atelectasis of lung parenchyma, focal hemosiderosis of the lower lobes of the lungs, secondary pulmonary hypertension, and focal intraalveolar hemorrhages.

On the 28th of September the results of a bacteriological examination of bronchial lavage showed the presence of Klebsiella pneumoniae sensitive to Imipenem, Ceftazidime, Ceftriaxone, Cefepime, Gentamicin, Amikacin, Cefoperazone, Polymyxin B and Tobramycin.

On the 29th of September was performed the transfusion red blood cells.

On the 6th of October the results of a bacteriological examination of bronchial lavage showed the presence of Klebsiella pneumoniae sensitive to Imipenem, Ceftazidime, Ceftriaxone, Cefepime, Gentamicin, Amikacin, Cefoperazone, Polymyxin B and Tobramycin. Ampicillin, amoxicillin, cefotaxime, tobramycin, amikacin resistant. Ceftazidime, ceftriaxone, cefepime, cefoperazone, cefpirom are moderately resistant.

On the 11th of October pleural drainage from the right pleural cavity was removed. The results of a bacteriological examination of bronchial lavage showed the presence of Streptococcus viridans sensitive to ampicillin, amoxicillin, cefotaxime, ceftriaxone, clindamycin, doxycycline, vancomycin, rifampicin, gatifloxacin and resistant to linezolid, and erythromycin. On the chest X-Ray there were positive dynamics due to the reduction of fluid volume in both pleural cavities. Focal infiltrative changes in the lungs were not detected.

In the early postoperative period, arterial blood gas measurements (3), electrolyte levels and level of glycemia were monitored and corrected every 2-4 hours, biochemical examinations (1) (2), blood coagulation test, CRP, PCT, tacrolimus concentration (4) and chest X-Ray daily.

Treatment in the postoperative period included:

- antibacterial therapy, which was selected according to the results of bacterial seeding
- antifungal therapy
- antiviral therapy
- analgesia
- volemic support
- maintenance of hemodynamics by administration of norepinephrine through a syringe dispenser with dose adjustment according to parameters.
- maintaining a negative water balance with furosemide for the first three days
- immunosuppressants
- prevention of thrombosis from the 2nd day - low molecular weight heparins in a prophylactic dose
- prevention of ulceration
- acetylcysteine

After a high-quality lung transplantation and qualified treatment in the postoperative period, the patient was discharged from the hospital on the 37th day of the postoperative period in satisfactory condition. The patient underwent a full course of rehabilitation and recommendations for further recovery.

CONCLUSIONS

Thus, based on the data of the world literature, as well as on the results of treatment on the basis of Municipal Non-Profit Enterprise " Lviv Emergency Clinical Hospital" we can conclude that lung transplantation is one of the most effective methods of treatment of patients with end-stage lung disease. This operation improves patients' quality of life, general well-being and ability to physical activity.

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CASE STUDY

NEUROFIBROMATOSIS TYPE I AND ITS DIAGNOSTIC CRITERIA: A CLINICAL OBSERVATION

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ABSTRACT

The aim – to consider the etiopathogenesis, the main clinical manifestations, diagnostic criteria of NF1, and present a clinical case from their practice. The paper analyzes the research findings in recent publications, focused on the studied issue using the methods of continuous sampling, synthesis and generalization, bibliosemantic evaluation and content analysis. In order to attract the attention of family physicians, neurologists, dermatologists, ophthalmologists, surgeons and other specialists, we present our own clinical observation of NF1.

The patient was examined using the methods of neurological examination, as well as other laboratory and instrumental methods of research. Early diagnosis and medical examination of patients with NF1 is crucial for predicting and improving the quality of life of patients. NF1 is a complex disease where the cooperation of doctors of different specialties is important. A favorable prognosis for patients is associated with the possibility of early diagnosis of malignant transformation and timely treatment.

KEY WORDS: neurofibromatosis type I, etiopathogenesis, diagnostic criteria, clinical manifestations, clinical case

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INTRODUCTION

Recklinghausen's neurofibromatosis or neurofibromatosis type I (NF1) is the most common disease in the group of phacomatosis or neurocutaneous syndromes. This is a diverse group of systemic diseases of hereditary nature, whose major clinical manifestations are pigmented, depigmented and vascular spots in combination with tumor-like neoplasms of the skin, blood vessels, central and / or peripheral nervous system [1-5].

NF1 is one of the most common monogenic human diseases, which occurs with a frequency of at least 1:3000-1:5000 of population, comprising up to 95% of all patients with NF [5-7]. NF1 is inherited by autosomal dominant type with high penetrance and variable expression of the pathological gene. Men and women are affected equally often [5, 8].

THE AIM

The aim of this research is to consider the etiopathogenesis, the main clinical manifestations, diagnostic criteria of NF1, and present a clinical case from their practice.

MATERIALS AND METHODS

The paper analyzes the research findings in recent publications, focused on the studied issue using the methods of continuous sampling, synthesis and generalization, bibliosemantic evaluation and content analysis. In order to attract the attention of family physicians, neurologists, dermatologists, ophthalmologists, surgeons and other specialists, we present our own clinical observation of NF1. The patient was examined using the methods of neurological examination, as well as other laboratory and instrumental methods of research.

REVIEW

Etiologically, NF1 is associated with a mutation in the NF1 gene, which is located on the long arm of chromosome 17 (17q11.2) and encodes the synthesis of neurofibromin protein. The latter is a suppressor of tumors, serving as a regulator of proliferation and differentiation of many cells. With the formation of pathological neurofibromin, there is an uncontrolled proliferation in individual neurons, glia cells, Schwann cells and melanocytes. The morphological substrate of neurofibromatosis are hamartomas, which are of ectodermal origin, located mainly in the nervous tissue and skin, and have blastomatous tendencies [2, 4, 5, 7].

According to the International Statistical Classification of Diseases and Related Health Problems, X revision, NF is a class XVII disease: "Congenital anomalies (malformations), deformities and chromosomal abnormalities", heading Q85 "Phacomatosis, not classified in other headings «[6].

Table I. Nondiagnostic Cutaneous and Extracutaneous Signs to Consider in Addition to the "Classical" Diagnostic Criteria when Evaluating Patients with NF1

Genetic analysis	Molecular analysis of the NF1gene
Cutaneous signs	Anemic nevus Juvenile xanthogranuloma Mixed vascular hamartomas and cherry angiomas Hypochromic macules "Soft-touch" skin Hyperpigmentation
Extracutaneous	Choroidal hamartomas Large head circumference and hypertelorism Unidentified bright objects on neuroimaging Cerebrovascular dysplasia, moyamoya Learning, speech and behavioral disabilities, headache and seizures Neoplasms

Table II. Clinical characteristics of the defined subtypes of neurofibromas

Neurofibroma Types	Location	Clinical Signs	Presentation
Cutaneous	Epidermis and dermis	Moves with skin, bluish tinge, local pruritus. Size: ~2 mm to 3 cm	Asymptomatic; most common type; presents in late teens
Subcutaneous	Deep to dermis	Skin moves over, firm and rounded feel, located along peripheral nerves. Size: ~ 3 to 4 cm	Tenderness on palpation; tingling in distribution of affected nerve
Nodular plexiform	Localized interdigitation with normal tissues	"Bag of worms" feel	May be present from birth, enlarges during the first decade of life
Diffuse plexiform	Infiltrate widely and deeply	Smooth slightly irregular skin thickening	-

When diagnosing NF1, it is necessary to use diagnostic criteria approved by the International Committee of Experts at the National Institute of Health (NIH) of the US (NIH – diagnostic criteria) in 1987 [7-15].

The diagnosis of NF1 (Recklinghausen's disease) is confirmed if a patient has at least 2 of the following signs:

1) six or more pigment spots (cafe-au-lait lesions) with a diameter of more than 5 mm in children of prepubertal age and more than 15 mm in postpubertal age;
2) two or more neurofibromas of any type or 1 plexiform neurofibroma;
3) freckles in the axillary or inguinal folds;
4) optic nerve glioma;
5) two or more Lisch nodules on the iris (hamartoma of the iris), which are detected during the study with a slit lamp;
6) dysplasia of the sphenoid bone or congenital thinning of the cortical layer of long bones with or without pseudoarthrosis;
7) the presence of NF1 in immediate relatives (parents, children, siblings) according to the above criteria.

These diagnostic criteria are highly specific for adults with NF1 [9].

Although these "classic" criteria for the diagnosis of NF1 are widely used and agreed upon, in recent years, additional new cutaneous and extradermal features have been described (Table I) [3].

It should be emphasized that these signs can occur in any combination, but none of them in itself is sufficient for the diagnosis of NF1 [8].

Skin manifestations are the most accessible diagnostic criteria and in most cases are the first symptoms of this multisystem disease. Signs of skin pigmentation – spots of color («café-et-lait» lesions), small pigment spots such as freckles in the inguinal and axillary folds (Crowe's symptom), generalized hyperpigmentation, tumors on the skin – neurofibromas are among the main manifestations of the skin syndrome in NF1 [4, 7, 9].

Pigment spots such as «café-et-lait» lesions of medium size (diameter 1-5 cm) are observed in 95% of patients with NF1; they appear either at birth (34%) or in childhood (66%) and are a classic symptom for NF1. They are monochrome spots from light white to dark brown, which are uniform in structure, round or oval in shape, with smooth edges and clear borders. Sizes can vary from a few millimeters to several centimeters, but diagnostically significant are spots with a diameter of more than 5 mm in children before puberty and more than 15 mm after puberty [5, 8, 9, 10]. In patients with NF1, melanocytes supersaturated with melanin and giant melanosomes are found in the areas of the spots [4, 5, 9].

Freckles are small hyperpigmented spots of a clearly rounded shape, 1-3 mm in diameter, with a more intense color than «café-et-lait» spots, brown in color. The

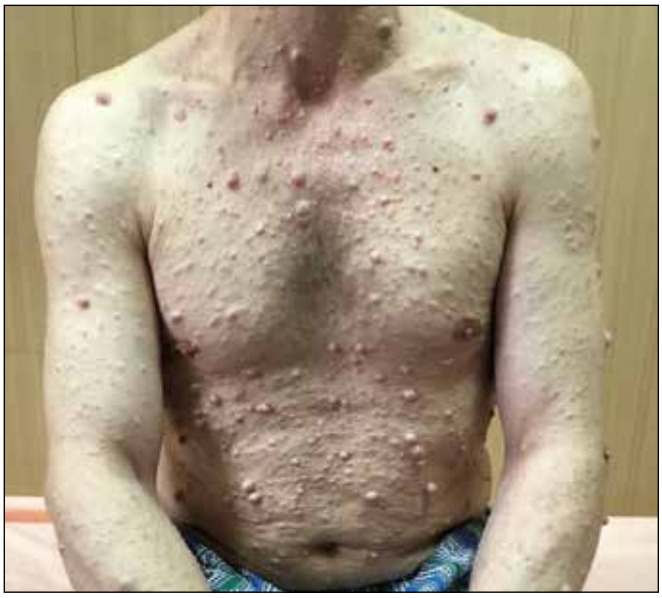

Fig. 1. Multiple cutaneous and subcutaneous neurofibromas on the anterior surface of the chest abdomen and upper extremities in patient V.

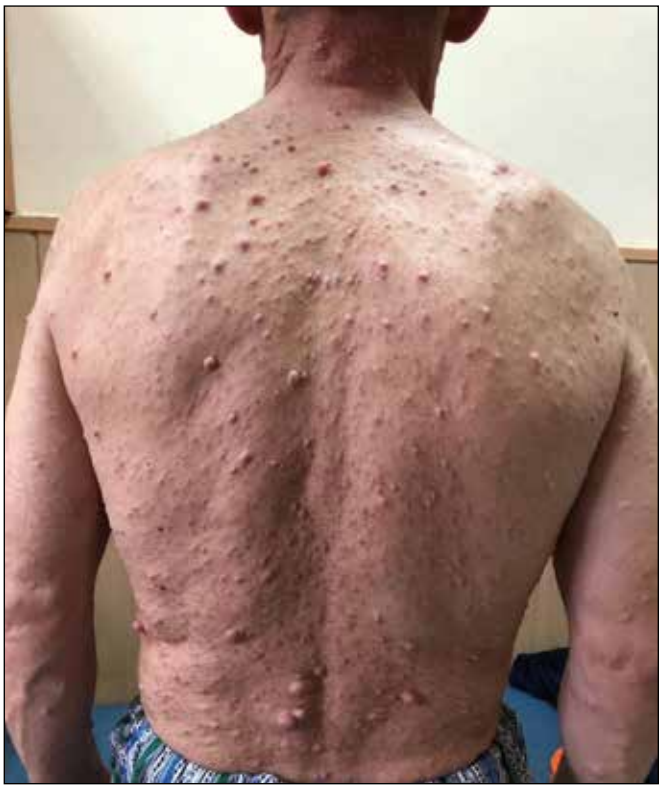

Fig. 2. Multiple cutaneous and subcutaneous neurofibromas on the back of patient V.

frequency of detection of freckles in NF1 varies from 21% to 93.7% [4, 5, 9].

Neurofibromas are the most characteristic manifestation of Recklinghausen's disease. They are benign formations that are derived from the nerve sheath of peripheral nerves, consisting of different types of cells: Schwann cells, fibroblasts, mast cells, endothelial cells, collagen fibers. Neurofibromas occur in the form of skin, subcutaneous

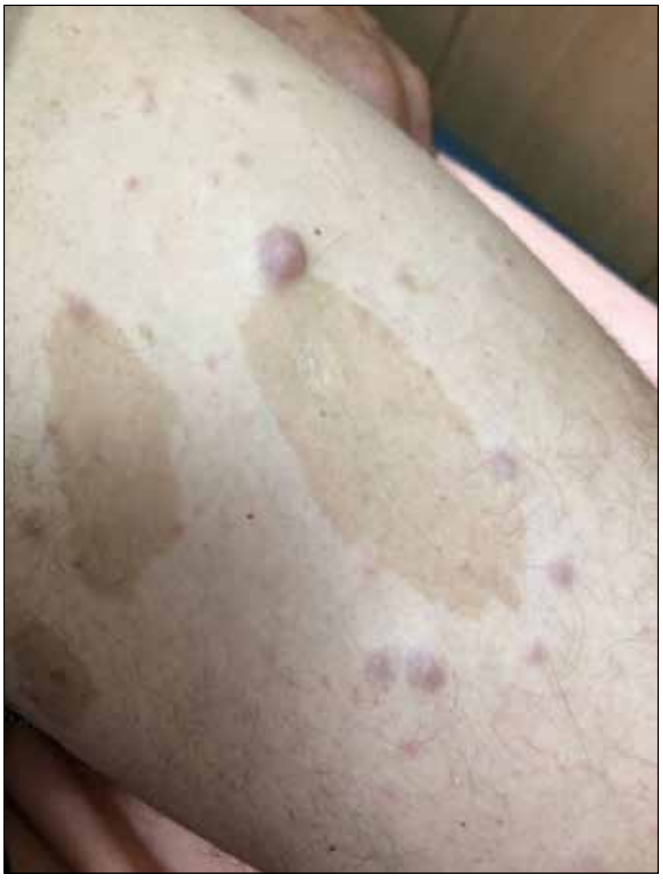

Fig. 3. Pigment spots cafe-au-lait lesions skin neurofibromas on the front and inner surface of the left thigh

and plexiform (nodular and diffuse) tumors [8, 12, 13]. Characteristics of each of them are presented in Table II.

Approximately 20% of patients have ophthalmic manifestations – Lisch nodules and optic nerve glioma [4, 8, 11]. Lisch nodules (iris hamartomas or melanocyte nevi) are one of the diagnostic criteria for the disease and are convex superficial clusters of pigment cells (from barely noticeable in size to 5 mm in diameter) on the iris, which can be seen in the study with a slit lamp. In children under 6 years of age, Lisch nodules are found in 15-20% of patients, and in adults – up to 95%. These nodules consist mainly of melanocytes, Schwann cells and fibroblasts. Optic nerve glioma is a benign tumor, rare in young children, more often manifested at the age of ten years in the form of a gradual decrease in vision. Optic nerve glioma occurs in 15-20% of patients with NF1 [7, 9, 10, 14-16].

Nervous system lesions in NF1 are of various nature – cognitive disorders of varying degrees (from mild to severe), attention deficit hyperactivity disorder, paralysis and paresis, syncopal paroxysms, epileptic seizures (focal, generalized), changes in the autonomic nervous system, headache, hearing and visual impairment [5, 7, 17-19]. It is very important to recognize and correctly interpret nonspecific neurological complaints, such as headache, in time [20]. The prognosis for patients with syncopal paroxysms depends almost entirely on the nature of the underlying disease [17].

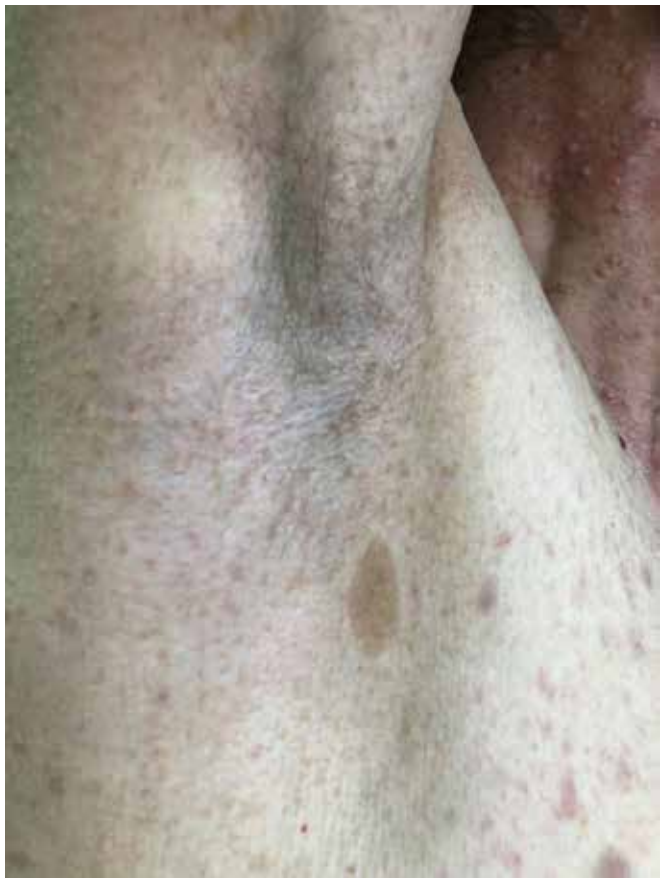

Fig. 4. Freckles in the axillary area

NF1 is characterized by additional clinical manifestations: endocrine disorders (pheochromocytoma, growth and puberty disorders); skeletal changes (scoliosis - up to 15%, chest deformity, spondylolisthesis, non-junction of vertebral arches, craniovertebral abnormalities, cranial asymmetry, pseudoarthrosis); cardiovascular manifestations (congenital heart defects, stenosis and aneurysm of the renal arteries, arterial hypertension), cerebral arteriopathy, etc. [5, 6, 9].

Primary diagnosis of NF1 should be performed by pediatricians, general practitioners of family medicine, as well as narrow specialists: neurologists, ophthalmologists, dermatologists, surgeons in the process of dynamic dispensary observation of the population. The leading role belongs to the fact that the process of development of clinical symptoms of NF is progressive, therefore, the dispensary supervision by doctors of different profiles of this group of patients and timely implementation of a set of additional diagnostic methods including CT and MRI of the brain and spinal cord are is essential [5, 7, 18].

The method of choosing tactics for patients on NF1 consists in observation and symptomatic treatment depending on the manifestations of the disease [6]. Surgical treatment is indicated for pain and increase in tumor size, compression or displacement of vital organs, suspected malignant tumor degeneration [4, 7]. Radiation therapy and chemotherapy are performed in the case of malignancy of tumors (from 3% to 20% of all cases of NF1) [7, 9, 21].

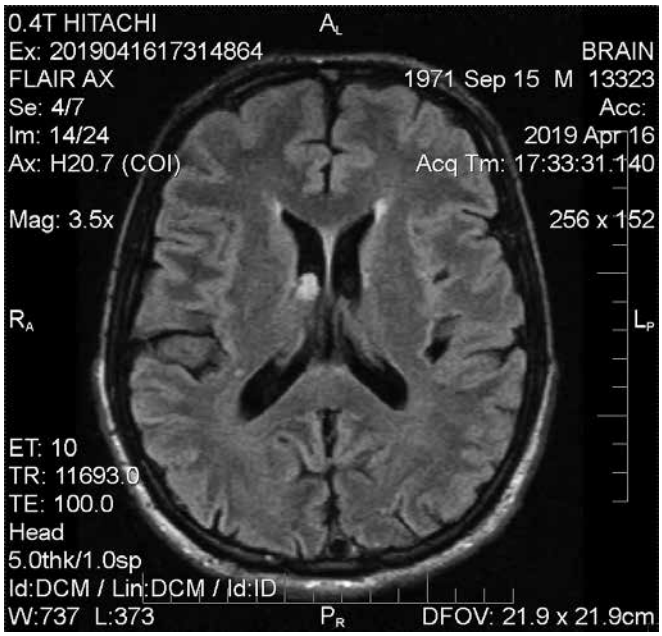

Fig. 5. MRI of the brain of patient V. (Apr 16, 2019)

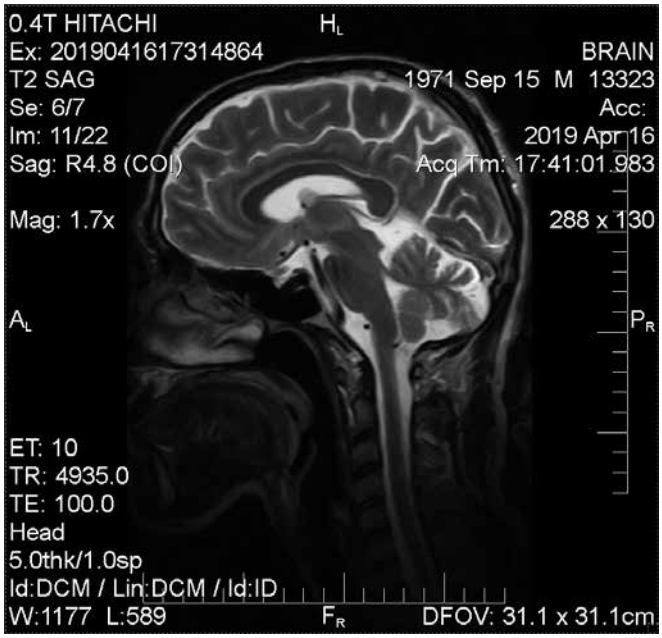

Fig. 6. MRI of the brain of patient V. (Apr 16, 2019)

The prognosis depends on the degree of manifestation of clinical signs. Malignant degeneration of neurofibromas is one of the important causes of death among patients with NF1 [4, 7, 9].

Due to the significant spread of this disease and the presence of severe and complicated forms in some patients, the study and description of cases of NF in different populations are extremely relevant [2].

CLINICAL CASE

In order to attract the attention of family physicians, neurologists, dermatologists, ophthalmologists, surgeons

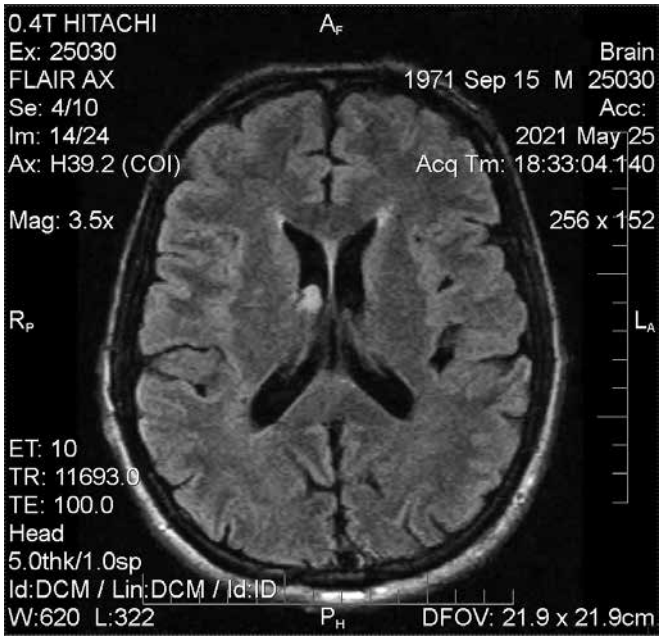

Fig. 7. MRI of the brain of patient V. (May 25, 2021)

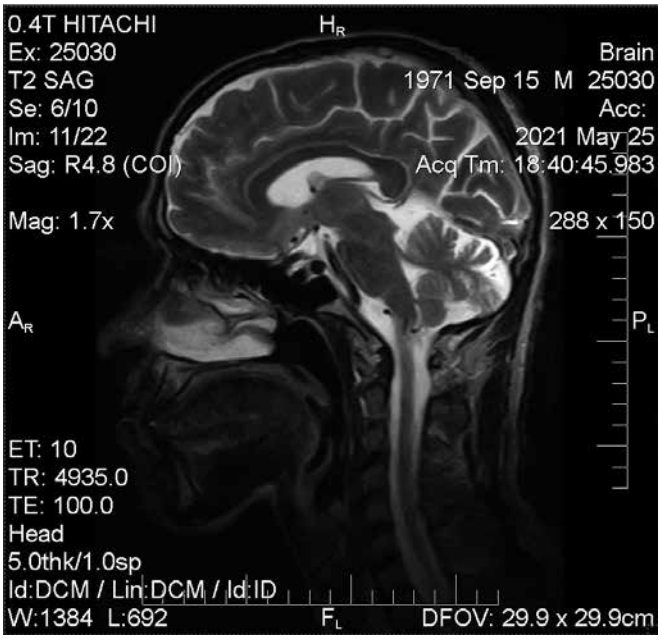

Fig. 8. MRI of the brain of patient V. (May 25, 2021)

and other specialists, we present our own clinical observation of NF1.

Patient V., 49 years old, first consulted a neurologist with complaints of periodic diffuse pressing headache, shakiness when walking, dizziness, increased blood pressure to 200/110 mm Hg, general weakness, memory loss, distraction, the presence of multiple tumor-like neoplasms on the torso, neck, upper and lower extremities, single neoplasms – on the face, which did not trouble the patient.

From the anamnesis of the disease: the patient considers himself to be ill from the age of 18, when tumor-like formations appeared on the trunk for the first time without a reason, which gradually increased in diameter and spread to other parts of the patient's body. In addition to the cosmetic defect, tumor-like neoplasms are painless, they do not trouble the patient. Pigment spots of the cafe-au-lait color appeared in the patient's childhood, he did not pay attention to them.

Life anamnesis: the patient has been suffering from hypertension for about 5 years. Hereditary history is not burdened.

Objective examination revealed the following abnormalities: multiple dome-shaped, soft, fleshy bluish-red and of normal skin color tumor-like formations of different sizes on the skin and / or in the skin thickness of the torso, neck, limbs (neurofibromas), which are painless on palpation. On easy pressing of rounded knots, there is a feeling of falling in of a finger (the phenomenon of the «bell push button») (Fig. 1, 2).

On the skin of the trunk and extremities, there are pigment spots (more than 6 spots) of the type of the cafe-au-lait color, which have different shapes and sizes (Fig. 3), in the axillary areas – small round pigmented spots with a color up to brown of the freckle type (Fig. 4).

In the neurological status: focal neurological symptoms are not detected. There are diffuse brisk deep reflexes, decreased abdominal reflexes, cognitive disorders (according to the MMSE scale – 27 points, indicating mild cognitive dysfunction in the patient).

Additional laboratory and instrumental methods of examination: general analysis of blood and urine, blood glucose within normal limits. In the biochemical analysis of blood: dyslipidemia with an increase in the fraction of atherogenic lipoproteins, coagulation parameters, the level of thyroid hormones – within normal limits.

Chest radiography – no pathology was detected.

ECG: sinus bradycardia. Normal position of the electrical axis of the heart.

Ultrasound of the abdominal cavity, adrenal glands: Liver cyst. Pancreatic cyst. Cysts of the right kidney.

Ultrasound of the thyroid gland: in the right and left lobes of the thyroid gland, there are multiple isoechoic nodes with cystic areas and the hydrophilic rim.

EEG: moderate diffuse changes in the bioelectrical activity of the brain by the type of disorganization of the main cortical rhythms. Paroxysmal activity and focal pathology at the time of examination were not registered.

On MRI of the brain without intravenous contrast (April 16, 2019): on a series of MR tomograms of the brain in sagittal, axial and frontal projections, in the projection of the anterior horn of the right lateral ventricle at the level of Monroe's orifice, there is an area of hyperintense MR-signal on FLAIR, weakly hyperintensive on T2BI, isointensive on T1BI with clear, uneven contours, irregular shape, conditional dimensions up to 10x10x9mm with a small diffusion restriction.

In the temporal areas, periventricular to the temporal horns of the lateral ventricles, near the hippocampus on both sides, there are areas of weakly hyperintensive MR-

Fig. 9. Lisch nodules on the iris

signal on FLAIR and T2BI with fuzzy, uneven contours, conditional sizes from 16x7mm to 33x16mm (Fig. 5, 6).

Conclusion: MR signs of the local area of the altered MR signal in the projection of the anterior horn of the right lateral ventricle (possibly the subependymal node), which requires dynamic monitoring; MR signs of areas of the changed MR signal symmetrically on both sides in the temporal lobes

On MRI of the brain with intravenous contrast (Dotavist 15 ml) (25.05.2021): there is a new area of the altered MR signal hyperintensive on FLAIR, weakly hyperintensive on T233, isointensive on T133 with clear, uneven contours, irregular in shape, irregular shape, 11x9x11mm in the right hemisphere of the brain periventricularly to the posterior parts of the body of the right lateral ventricle at the border of the temporal and parietal lobes. In comparison with the MRI data from 16.04.2019 apart from this – MR-pattern without dynamics. After intravenous contrast enhancement, the absence of pathological accumulation of contrast agent is determined (Fig. 7, 8).

Ophthalmologist's consultation: In the fundus – the optic discs are pale pink, clear borders, retinal arteries are constricted, veins are moderately dilated. Visual acuity of both eyes = 1.0. During the examination with a slit lamp, small Lisch nodules on the iris of both eyes were found (Fig. 9).

Consultation of the ENT specialist: no pathology not detected.

Cardiologist's consultation: Hypertensive disease of the II degree, grade 2, risk III. Coronary heart disease: Diffuse cardiosclerosis, sinus bradycardia. Heart failure of the I grade, functional class II.

DISCUSSION

Thus, as a result of the examination of the patient, there are at least four criteria for the diagnosis of NF1:

- the presence of more than 6 pigment spots of the café-et-lait color with a diameter of more than 15 mm;
- in the axillary areas, there are small pigmented spots of round shape with a color up to brown of the "freckles" type;
- Lisch nodules on the iris of both eyes, which were detected during the study with a slit lamp;
- multiple neurofibromas on the skin and in the skin thickness of the torso, neck, limbs.

In addition to the «classic» criteria for the diagnosis of NF1, the patient had additional extradermal signs: the areas of weakly hyperintense MR-signal on T2-weighted mode are determined on the brain MRI; mild cognitive disorders, headache.

The presence of hyperintensive foci is one of the natural manifestations of NF1 in patients, which is visualized on the brain MRI in T2-weighted mode. These are hyperintensive foci, whose clinical significance is unknown, which is why they were called unidentified (unestablished) bright objects. They are found in the amount of 60% to 93% in children with NF1. The association between unidentified bright objects and the retardation of children with NF1 in development has been established [3, 5, 6].

According to MRI of the brain, the presented patient has disease progression, which is manifested by the appearance of a new area of the altered MR signal in the right hemisphere of the brain. The patient is recommended to consult a neurosurgeon and oncologist to determine further management of the patient (taking into account the changes in the brain MRI) and constant monitoring by a neurologist, ophthalmologist, dermatologist, surgeon, gastroenterologist, nephrologist, and endocrinologist.

It is essential to remember that the process of development of clinical symptoms in NF1 is dynamic – it is slow, but continuously progressive [5, 7, 18].

CONCLUSIONS

NF1 is a complex problem of modern medicine, due to the significant phenotypic and clinical variability of the disease, age-dependent onset, hereditary nature, the complexity of its diagnosis and treatment, reduced quality of life of patients. The most prognostically unfavorable complication of NF1 is malignant degeneration of neurofibromas.

Therefore, early diagnosis and medical examination of patients with NF1 is crucial for predicting and improving the quality of life of patients. NF1 is a complex disease where the cooperation of doctors of different specialties is important. A favorable prognosis for patients is associated with the possibility of early diagnosis of malignant transformation and timely treatment.

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CASE STUDY

LARGE INTESTINE OBSTRUCTION CAUSED BY PSEUDOPOLYPOSIS IN THE COURSE OF ULCERATIVE COLITIS – A CASE REPORT

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ABSTRACT

Ulcerative colitis is an inflammatory bowel disease. Opposite to Crohn's disease, it affects only the mucosa and submucosa of the large intestine. The disease can have episodes of flares and remissions. Endoscopy studies often disclose pseudopolyps, which are perceived as non-neoplastic and usually do not require removal. Rarely pseudopolyps can become big or even gigantic, leading to abdominal pain, bowel movement dysfunction, or even bowel obstruction. We present a case of a 37-year-old male patient with ulcerative colitis who was operated on because of a gigantic pseudopolyp causing bowel obstruction.

KEY WORDS: ulcerative colitis (UC), pseudopolyposis, bowel obstruction

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INTRODUCTION

We distinguish mainly ulcerative colitis (UC) and Crohn's disease among inflammatory bowel diseases. Both diseases are autoimmune and genetic. Although their etiology and pathogenesis are still not fully elucidated, it is noted that environmental factors play a significant role in their etiology. Ulcerative colitis is a chronic inflammatory disease of the large intestine. In its clinical course, we observe periods of exacerbation (disease relapses) and remissions. The main symptoms are watery-bloody diarrhea (up to 20 times a day), abdominal pain, painful pressure on the stool, fever, and progressive cachexia [1]. Although the primary treatment in most cases is a conservative one (appropriate diet and pharmacological treatment), it is estimated that approximately 15-20% of patients require surgical intervention during that treatment. The diagnosis is usually made based on characteristic clinical symptoms, endoscopic examination results, histopathological examination of specimens obtained during colonoscopy, and the bacterial culture of the stool.

The main complications of UC are dehydration, gastrointestinal bleeding, acute toxic megacolon, and colorectal cancer. The risk of colorectal cancer is 1-3% after ten years of disease and increases 1-2% annually. In the further course of UC, we can observe primary obstructive cholangitis, nephrolithiasis, inflammation of the skin, joints, and eyes, as well as colon perforation [2].

Bowel obstruction is a rare UC complication. It is caused by pseudopolyposis of the large intestine, especially when a huge pseudopolyp is present, i.e., the diameter exceeds 15 mm, obstructing the intestinal lumen [3, 4]. Despite their size, these polyps are usually benign and are more common in UC than in Crohn's disease [4]. Some of them respond to conservative treatment, with symptom relief and a reduction in the size of the pseudopolyp [5]. In the case of no improvement during conservative treatment and with symptoms of complete obstruction, surgery seems to be the only correct solution.

CASE REPORT

A 37-year-old patient treated for UC for many years was admitted to the Department of General Surgery and Proctology of the Solec Hospital due to persistent abdominal pain, mainly in the mid and right lower abdomen, and persistent loose, watery stools (up to 8 a day) lasting for many months. Despite the conservative treatment, mesalazine at a dose of 1g three times a day, large doses of immunomodulating drugs, and glucocorticosteroids, no clinical improvement was achieved. Moreover, the patient also reported nausea, abdominal distension, difficulty in flatulence, and deterioration of well-being. The patient was treated chronically due to arterial hypertension. He neglected food and drug allergies and was a tobacco smoker for many years. He was treated three times in the hospital:

1. Hospitalization in December 2017 due to UC exacerbation and Clostridium difficile infection. The abdominal computed tomography scan showed no signs of gastrointestinal obstruction. Metronidazole was administered, and clinical improvement was achieved.
2. Another hospitalization in January 2018, during which the patient underwent gastroscopy and colonoscopy. The colonoscopy revealed mucosa of the ascending colon, transverse colon, descending colon, sigmoid colon, and

rectum without a vascular pattern with numerous erosions, ulcerations, paving, and pseudopolyps, especially on the right side of the colon.

3. The third hospitalization took place in May 2018. Again, a colonoscopic examination was performed. The apparatus was inserted into the transverse column and revealed a large polypoid lesion of the colon, narrowing the lumen of the large intestine. No signs of mucosal dysplasia were found in the samples taken from the lesions.

The patient was in good general condition, without cardio-respiratory compromise on admission. The abdomen on examination was soft but tender on palpation, mainly in the right lower abdomen. No peritoneal symptoms were found. The additional laboratory tests showed: anemia of 10.7 g/dL, and other blood counts were normal (June 27, 2018)

Due to the advancement of polyposis and symptoms of bowel obstruction, the patient was qualified for surgery. On June 28, 2018, the patient underwent surgery. Laparotomy was performed. The colon was excised to form the rectal stump using the Hartmann method, and the final ileostomy was performed. Drainage of the peritoneal cavity and subcutaneous tissue was applied. The postoperative period was uneventful. The patient was discharged home in good general condition on the 5th postoperative day.

The histopathological examination of 07.July 2018 year revealed the following findings. Macroscopically it was a fragment of the large intestine (70 cm) with a fragment of the small intestine (3 cm) and an appendix (6 cm). At a distance of 11 cm from the proximal margin and 37 cm from the distal margin, polyp with the largest dimension of 17 cm was located. Microscopically it showed numerous pseudopolyps with atrophy and hyperplasia of the crypts, abundant various-cell inflammatory infiltrates, numerous crypt abscesses, cryptitis-like features, and places with purulent-fibrinous masses on the surface. In individual glands, low-grade dysplasia was shown. Neither high-degree dysplasia nor cancer was found in the examined specimens.

DISCUSSION

The risk of developing colorectal cancer in UC is approximately 1-3% after ten years of the disease. This risk increases 1-2% annually in the following years. Pseudopolyps are one of the late complications. They are formed in the large intestine throughout the UC course. Dysplasia of various stages appears in the diseased intestinal mucosa and can be an early symptom of cancer development. Colon pseudopolyposis, together with foci of dysplasia, is an indication for careful monitoring of the patient in terms of the risk of developing colorectal cancer. It is recommended to perform colonoscopic examinations with the collection of numerous sections of the mucosa from macroscopical lesions at least every two years up to 10 years of the disease, and after ten years - the examination should be performed every year. The diagnosis of dysplasia, especially of high-degree, or the detection of an adenocarcinoma focal point is an absolute indication for surgery. Most often, dysplastic changes are disseminated, and they characterize discontinuous growth. Some people recommend surgery also in cases of mild dysplasia if the disease lasts more than 15 years and covers the entire colon. The rationale for doing so is a high risk of overlooking or omitting the sites where dysplastic changes were found. The presence of low-grade dysplasia justifies a colonoscopy every six months [1, 2]

In this patient, the indications for surgery were as follows:

- a large pseudopolypoid lesion in the transverse column which resulted in a critical impairment of intestinal passage;
- disseminated low-grade dysplastic changes in the mucosa of the large intestine.

The patient was recommended to perform a Hartmann colostomy. The rectum was left behind, and its proximal stump was closed "deaf". The end segment of the ileum was led to the abdominal wall in the form of an end ileostomy. In the postoperative period, the subsequent stages of treatment were discussed with the patient, considering the following management options:

a. rectal excision with an ileal reservoir and anastomosis between the intestinal reservoir and the anal canal (reconstruction of the gastrointestinal tract);

b. total rectal resection and permanent ileostomy;

c. endoscopic and histological monitoring of the rectum for several years, leaving an ileostomy.

During the follow-up (August 2021), the patient felt well. The stoma was working properly. Endoscopy of the rectal stump was scheduled. The patient did not choose to restore continuity or remove the rectal stump. He stays under continuous observation.

CONCLUSIONS

This rare case (pseudopolyp-induced obstruction) is another confirmation that UC, due to its course (exacerbations and remissions) and the nature of possible complications - both immediate life-threatening and long-term (dysplasia, pseudopolyps, carcinogenesis), is a disease that requires interdisciplinary care and systematic continuous endoscopic surveillance.

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Conflict of interest
The Authors declare no conflict of interest.

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Pallas Kliniken

Kliniki Pallas specjalizują się w okulistyce i medycynie estetycznej. Zatrudniamy ponad 350 pracowników w 18 lokalizacjach i jesteśmy jednym z wiodących świadczeniodawców w Szwajcarii. Poprzez innowacje stale rozwijamy naszą ofertę usług w tych dziedzinach. Czy to poprzez nowe metody leczenia, sprzęt, dodatkowe lokalizacje i współpracę, a może poprzez współpracę z Państwem w najbliższej przyszłości?

W celu uzupełnienia istniejącego zespołu w naszej grupie w lokalizacjach **Olten, Aarau i Solothurn** poszukujemy osoby z inicjatywą i niezależną osobowością na stanowisko

specjalista oftalmologii (k/m/i) 60–100%

Główne obowiązki

Specjalista oftalmologii w naszych placówkach w Olten, Aarau lub Solothurn zapewnia profesjonalną obsługę medycznych konsultacji zachowawczych. Osoba ta będzie kompetentnie wykorzystywać swoje umiejętności we współpracy z obecnymi lekarzami specjalistami. Do jej obowiązków będzie należało zapewnienie naszym pacjentom optymalnej opieki. Wraz ze zgranym zespołem będzie świadczyć usługi medyczne na najwyższym poziomie. Będzie korzystać z szerokiej sieci wybitnych lekarzy, ciągłych szkoleń wewnętrznych i zewnętrznych oraz nowoczesnego środowiska pracy.

Profil kandydata

- Specjalizacja w dziedzinie oftalmologii
- Kilkuletnie doświadczenie w dziedzinie oftalmologii
- Przedsiębiorcze myślenie i działanie zorientowane na sukces i cel, z wysokim zrozumieniem jakości i obsługi
- Wysoka inteligencja emocjonalna i odporność w kontaktach z pacjentami, pracownikami oraz innymi partnerami wewnętrznymi i zewnętrznymi
- Umiejętność szybkiego rozpoznawania problemów i samodzielnego opracowywania rozwiązań
- Wybitne kompetencje doradcze w języku niemieckim, wyrażane w mowie i piśmie w sposób zrozumiały i adekwatny do adresata

Nasza oferta

W ramach udzielania konsultacji udostępniamy nowoczesną infrastrukturę, w której można profesjonalnie leczyć pacjentów, zarówno ambulatoryjnie, jak i stacjonarnie. Zapewniamy optymalne i jak najlepsze wsparcie podczas pracy. Oprócz uregulowanych godzin pracy (brak dyżurów nocnych i niedzielnych), które pozwalają na spędzanie czasu z rodziną i czas wolny, oferujemy także możliwości szkoleń wewnętrznych i zewnętrznych. Chętnie udzielimy dodatkowego wsparcia podczas stawiania pierwszych kroków w Szwajcarii.

Wykorzystując swoje wieloletnie doświadczenie, innowacyjność, a przede wszystkim umiejętność aktywnego słuchania, mogą Państwo naszym pacjentom pozwolić odczuć wyraźną różnicę.

Szukają Państwo wszechstronnej i ciekawej pracy w rodzinnej grupie przedsiębiorstw? Prosimy o przesłanie swojej aplikacji.

Dodatkowych informacji udziela pan Melvin Fankhauser, HR Recruiter/doradca HR ds. lekarzy, tel. +41 58 335 31 84 lub e-mail: melvin.fankhauser@pallas-kliniken.ch

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