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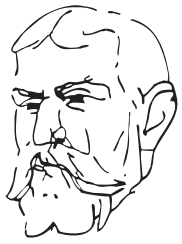

Memory of
dr Władysław
Biegański

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ORIGINAL ARTICLE

IS THERE AN ASSOCIATION BETWEEN HEALTH LITERACY AND ADHERENCE TO THE MEDITERRANEAN DIET? A CROSS-SECTIONAL STUDY IN GREECE

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ABSTRACT

The aim: To evaluate the relationship between adherence to the Mediterranean diet and health literacy and to find possible differences based on the demographic characteristics of the participants.

Materials and methods: A cross-sectional study was carried out with a convenience sample from the Greek general population (n=203). The questionnaires were distributed through the Google Forms platform, while some of them were given on hand. The questionnaire used included demographic information, questions about the participants' health status and nutrition habits and the Health Literacy Scale (HLS - EU-Q16) and the MedDietScore. The survey was conducted June – July 2022. The program SPSS v. 26.0 was used for the analysis of data.

Results: The mean score in HLS-EU-Q16 was 12.08 (SD=4.17) (15.8%=inadequate level, 30% =problematic level, 54.2%=sufficient level). The mean score in Med DietScore was 31.54 (SD=4.84) and the majority (72.4%) had medium adherence to the Mediterranean diet. Concerning the HLS-EU-Q16, women and those who had not children, were not patients and used the Internet for finding health information had higher score. In contrast, widows/ers and those with elementary education had lower score. With regard to the Med DietScore, those who were patients and had normal weight had higher score than the rest categories. A negative correlation was found between age-HLS-EU-Q16 (r=-.605, $p<.01$) and between BMI-Med DietScore (r=-.142, $p<.05$).

Conclusions: Most of the participants had sufficient level of health literacy and medium adherence to the Mediterranean diet. Furthermore, there was not a significant correlation between adherence to the Mediterranean diet and health literacy. Several demographic and medical characteristics impact the health literature level and the adherence to the Mediterranean diet. Future research is suggested to further evaluate the results of this study.

KEY WORDS: Health literacy, Mediterranean diet, adherence, association, demographics, differences

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INTRODUCTION

Health literacy is a significant factor in health care, health promotion, disease prevention and policy making. Everyone needs health literacy skills to read health information, to discuss, to participate in research studies, to use medical tools in everyday health care etc [1].

This concept is defined as "*the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions*" [2]. It is a determinant of health and low levels of health literacy are connected to risky behaviors, less healthy choices, riskier behavior, bad health status and longer hospitalization [3].

Health literacy levels vary across cultures, states and settings and are lower in persons with lower income and education and in older ages [4-5]. It has also been found that men tend to have lower levels of health literacy than women [6].

A great number of people uses the Internet for finding health information, eg. research has shown that 61% of the adults in the USA have searched for health/medical information on the Internet [7]. Diaz et al. examined 512 patients and found that 53.5% of them used the Internet to find medical information [8]. Those who used it were more educated and had higher incomes. Concerning gender differences, Bujnowska-Fedak found in Poland that women were at a slight advantage, with the exception of the oldest age group [9].

The Mediterranean diet is one of the most widely described dietary patterns in scientific literature [10]. It is a general term used to describe the dietary pattern of persons living in the

countries along the coast of the Mediterranean Sea, including Greece, Spain, Italy, southern France and parts of the middle east [11]. It is primarily a plant-based diet and is characterized by high intakes of fruits and vegetables, nuts, grains, fish, legumes, seafood, extra virgin olive oil, and a moderate intake of red wine [10]. The suggested numbers of portions for these foods are represented as a diet pyramid and those consumed in greatest quantities appear in the basis of the pyramid.

The Mediterranean diet is considered as one of the healthiest dietary patterns worldwide. Various studies have shown that adherence to it is associated with improvement in longevity, lower risk of mortality and with low incidence of cancer, cardiovascular and metabolic disease [10]. It has also been associated with reduced development of other health issues like kidney stones, osteoporosis, inflammatory bowel disease etc [11].

Demographic differences in the adherence to the Mediterranean diet haven't been extensively examined. In Obeid's et al. systematic review, the majority of the studies reported low/moderate adherence and there weren't clear differences across sex and age groups [12].

To the best of our knowledge, the relationship between adherence to the Mediterranean diet and health literacy is a neglected area of research. Various studies have evaluated the relationship between health literacy and healthy diet or nutrition generally and have shown a positive association between health literacy and healthy diet [13-14]. However, we found only one study that has examined the relationship between adherence to the Mediterranean diet and health literacy. In this study, conducted in two Italian universities with a sample of undergraduates from healthcare and non-healthcare departments, significant associations were found between health literacy and adherence to the Mediterranean diet. Moreover, the educational field and adherence to the Mediterranean diet were shown to be predictors of health literacy [15]. (Gallè et al., 2020).

THE AIM

The aim of this study was to investigate the relationship between adherence to the Mediterranean diet and health literacy and to evaluate possible differences in them based on the demographic characteristics of the participants. It was hypothesized that:

- Health literature is correlated with the adherence level to Mediterranean diet (hypothesis 1).
- There are differences in health literature across the demographic characteristics of the participants (hypothesis 2).
- There are differences in the adherence to the Mediterranean across the demographic characteristics of the participants (hypothesis 3).
- The age of the participants is associated with the health literacy and with the adherence to the Mediterranean diet (hypothesis 4).
- The Body Mass Index is associated with the health literacy and with the adherence to the Mediterranean diet (hypothesis 5).

MATERIALS AND METHODS

DESIGN

This was a cross-sectional study with a convenience sample of 203 people from the Greek general population. The questionnaires were distributed through the google forms platform, while some of them were given on hand. In this case, the participants took the questionnaire in their home, completed it and gave or posted it back to the researcher. The vast majority of the questionnaires (81.28 %) were completed online.

The eligibility criteria were the following: 1) men-women with ability to understand/respond to the questionnaire; 2) ability to understand the Greek language; 3) willingness to participate voluntarily in the research. Persons who were unable to respond to the questions and those who did not wish to participate voluntarily were excluded from the study.

A composite questionnaire was used including demographic data, information about the participants' health status and nutrition habits and the Health Literacy Scale ((HLS -EU-Q16) and the MedDietScore. The duration of the study was two months (06-07.2022).

PARTICIPANTS

According to the eligibility criteria, 203 people from the Greek general population participated in the study. Their mean age was 56 years (M=56.2, SD=18.5, Min=18, Max=93, Range=75). The rest demographic data of the participants are shown in table I.

MEASURES

DEMOGRAPHIC AND MEDICAL INFORMATION

Participants answered questions about their age, gender, job, marital status, level of education and reported if they have children. Concerning the medical information, they reported if they suffered from a disease, if they had ever taken medication for high blood pressure, if they had ever high blood sugar values and if any of their relatives had been diagnosed with diabetes mellitus. With regard to their health behaviors, they reported if they had an everyday physical activity (at least 30 minutes) and if they searched in the Internet to find health or medical information. Finally, they were asked about their waist circumference and about their weight and height, in order to calculate their Body Mass Index (BMI).

EUROPEAN HEALTH LITERACY SURVEY QUESTIONNAIRE (HLS-EU-Q16)

It is a self-reported questionnaire developed by the HLS-EU Consortium and evaluates health literature [16-17]. More specifically, it addresses difficulties in accessing, understanding and appraising information to tasks related with health promotion and disease prevention eg."how

Table I. Participants' demographic data

	N	%
Sex		
Man	80	39.4
Woman	123	60.6
Level of education		
Elementary school	57	28.1
Secondary school	12	5.9
High school	40	19.7
Vocational Training Institute	6	3.0
University student	49	24.1
University graduate	10	4.9
MA/Msc holder	28	13.8
Phd holder	1	0.5
Job		
Civil servant	50	24.6
Private employee	32	15.8
Freelancer	26	12.8
Unemployed	12	5.9
Pensioner	60	29.6
Student	3	1.5
Farmer	3	1.5
Housewife	15	7.4
Marital Status		
Single	37	18.2
Married	130	64.0
Widow/widower	29	14.3
Divorced/separated	5	2.5
Partnered	2	1.0
Having children		
Yes	154	75.9
No	49	24.1
Place of residence		
Town	22	10.8
City	104	51.2
Village	77	37.9

easy would you say it is to find information on treatments of illnesses that concern you?"[18]. It consists of 16 items rated in a 4-point Likert scale (very or fairly difficult, fairly or very easy) and a "no answer/don't know" item. The "difficult" categories are scored as 0 and the "easy" as 1 [19]. Scoring varies between 0 (no health literacy) and 16 (high health literacy), establishing the following three levels of health literature: inadequate (0-8), problematic (9-12) and sufficient (13-16) [16]. In this study the Greek translation of the questionnaire was administered [20] and the internal consistency relianility was excellent (Cronbach's α=.903.)

THE MEDDIETSCORE

It is an index that estimates the adherence to the Mediterranean diet. It includes the consumption of the following eleven food groups during the last week: whole grains, fruits and juices, vegetables and salad, legumes, potatoes, fish and soup, full-fat dairy, red meat and its products, poultry, olive oil in cooking and consumption of alcohol. The frequency of consumption is measured in portions per week and every item is rated in a 6-point scale: 0=no consumption, 1=1-6 portions/week, 2=7-12 portions/week, 3=13-18 portions/week, 4=19-31 portions/week,

Table II. Means and differences between the demographic categories and the HLS-EU-Q16 and MedDietScore

	HLS-EU-Q16	*p*	MedDietScore	*p*
Gender				
Man	11.25	***.011***	33.43	NS
Woman	12.63		32.60	
Marital Status				
Single	14.05		32.65	
Married	12.37	***<.001***	32.94	NS
Widow/widower	7.97		33.66	
Divorced/separated	13.20		31.40	
Partnered	14.00		30.50	
Level of education				
Elementary school	8.07	***<.001***	33.44	NS
Secondary school	12.42		34.08	
High school	12.88		32.18	
Vocational Training Institute	12.17		34.83	
University student	14.40		36.40	
University graduate	14.59		32.61	
MA/Msc holder	14.00		31.79	
Phd holder	12.00		33.00	
Having Children				
Yes	11.51	***<.001***	32.80	NS
No	13.90		33.33	
Being patient				
Yes	10.72	***<.001***	33.43	***.039***
No	13.76		32.31	
BMI categories				
Normal weight	12.51	NS	33.80	***.046***
Overweight	11.66		32.95	
Obese	12.13		31.80	
Use of internet for finding health information				
Yes	13.99	***<.001***	32.50	NS
No	9.38		33.52	

Note:
NS= No significant

5=>32 portions/week. Items concerning red meat and its products, poultry and full-fat dairy are reversed scored. The possible score ranges from 0 to 55 and higher values indicate greater adherence. It can also be divided in categories: 0-25=low adherence, 26-35=medium adherence, 36-55=high adherence. In this study the Greek translation of the index [21] was administered and Cronbach's a was .601.

DATA ANALYSIS

The statistical analysis was performed by the program SPSS 26.0. The normality of continuous variables was examined by the Kolmogorov-Smirnov test. Reliability analysis was performed by means of Cronbach's alpha. The analysis included at first the descriptive statistics. The linear correlation among quantitative variables was investigated by Pearson's correlation coefficient. Statistically significant differences between two groups or more than two groups were evaluated by t-test for independent samples and ANOVA test. Multiple comparisons in ANOVA were performed by means of Bonferroni correction. The *p*-value was set to 5%.

ETHICS

All participants who took the questionnaire on hand signed an informed consent. The researcher informed them in

Table III. Differences in the use of internet for finding health information

		Use of internet for finding health information		Total	*p*
		Yes	No		
Gender	Man	39	41	80	p<.001
	Woman	80	43	123	
	Total	119	84	203	
Being patient	Yes	45	67	112	p<.001
	No	74	17	91	
	Total	119	84	203	
Having Children	Yes	77	77	154	p<.001
	No	42	7	49	
	Total	119	84	203	

Table IV. Correlations

	HLS -EU-Q16	MedDietScore
Age	-.605**	.045
Body Mass Index	-.109	.142*
HLS-EU-Q16	1	-.072

Note: ** p< .01
* p< .05

detail about the aim of the study and gave them assurances of confidentiality and anonymity. The researcher also assured them that the collected data would be used only for the study's purposes. In the case of online completion, they read an information sheet and then clicked the "Click here to proceed" button to give their consent. If they wanted to quit, they had the option to withdraw by closing their browser. All of them participated voluntarily and no compensation was given.

RESULTS

The mean waist circumference was 96.45 cm (SD=19.27, Min=45, Max=160, Range=115) and the mean Body Mass Index was 27.19 (SD=4.8, Min=18.37, Max= 42.69, Range = 24.32). The majority were overweight (37.9%) or had normal weight (34.5%) and 27.6% were obese.

With regard to the participants' health status, 55.2% of them suffered from a disease, 20.2% had high glucose values in their blood tests and 44.8% received medication for high blood pressure.

A percentage of 35.5% had close relatives diagnosed with diabetes mellitus, while in 13.3% there was such a diagnosis in other relatives.

Concerning their exercise and nutrition habits, 51.2% of them had at least 30 minutes of exercise per day and 76.8% consumed fruits, vegetables and wholegrain bread in a daily basis. Most of them (58.6%) used the Internet to find health information.

The mean score in HLS-EU-Q16 was 12.08 (SD=4.17, Min=0, Max=16, Range=16). Concerning the level of Health Literature, 15.8% had inadequate level, 30% problematic level and 54.2% had sufficient level.

The mean score in MedDietScore was 31.54 (SD=4.84, Min=22, Max=46, Range=24). The majority of the participants had medium adherence (72.4%) and about one in four (25.6%) high adherence to the Mediterranean diet. In contrast, only 2% had low adherence.

No significant differences were found in the adherence to the Mediterranean diet across the health literacy level (inadequate etc).

As far as the HLS-EU-Q16 concerns, the following significant differences were found (table II):

– women had higher score than men (t= -2.32, df= 201, *p*= .011).
– those who hadn't children had higher score than those who had children (t=-3.60, df=201, *p*=<.001).
– those who weren't patients had higher score than those who were patients (t= -5.52, df=201, *p*=<.001).
– those who used the Internet for finding health/medical information had higher score than those who didn't use it for this reason (t= 9.24, df=201, *p*=<.001).

According to one-way ANOVA, there was a significant effect of marital status on HLS-EU-Q16 Score, F (4, 198) = 11.46, *p*=<.001, η_p^2=.19. Widows/widowers had lower score than single, married, partnered, separated or divorced.

In addition, there was a significant effect of educational level on HLS-EU-Q16 Score, F (8, 194) = 17.91, *p*=<.001, η_p^2=.43. Those who had completed the elementary school had lower score than the participants of the rest educational categories.

With regard to the MedDietScore, the following significant differences were found (table II):
– Patients had higher score than those who weren't patients (t= 1.78, df=201, p=.039).
– According to one-way ANOVA, there was a significant effect of BMI on MedDietScore, F (2, 200) =3.14, p=.046, η_p^2=.03. Those with normal weight had significantly higher score than obese participants. However, this effect was very small according to the effect size index.

With regard to the use of Internet for finding health/medical information, the following significant differences were recorded:
– women used the Internet more frequently than men for this reason (x^2=5.30, df=1, p<.001).
– those who weren't patients used the Internet more frequently than patients for this reason (x^2=35.032, df=1, p<.001).
– those who had children used the Internet more frequently than those who hadn't children for this reason (x^2=19.547, df=1, p<.001).

The age of the participants was strongly and negatively correlated with the HLS-EU-Q16 score (r=-.605, p<.01). Furthermore, their BMI was negatively associated with MedDietScore (r=-.142, p<.05). That is, the older people present lower health literature and those with higher BMI present lower adherence to Mediterranean diet. No other significant correlations were found (table IV).

DISCUSSION

This study aimed to assess the relationship between health literacy and adherence to Mediterranean diet in a sample of the general population in Greece. A second aim was to examine the differences in health literacy and adherence to Mediterranean diet across the several demographic and medical characteristics. According to the literature review very few studies have investigated this relationship, highlighting the novelty of the present study.

The main results showed that the majority of the participants had sufficient level of health literacy and medium adherence to the Mediterranean diet. No significant differences were found in the adherence to the Mediterranean diet based on the health literacy level (inadequate, problematic etc).

Furthermore, health literacy was not significantly related to adherence to the Mediterranean diet. Consequently, hypothesis 1 was not confirmed. This finding is not consistent with that found by Gallè et al., who found significant associations between adherence to the Mediterranean diet and health literacy in undergraduates from healthcare and non-healthcare courses in Italy [15].

In contrast, hypothesis 2 was confirmed and several differences in health literature were found based on the demographic characteristics of the participants. More specifically, women, participants who hadn't children and weren't patients had higher score. With regard to the gender, other researchers have also found that women have higher health literacy levels than men [6].

Moreover, widows/ers had significantly lower score than those who were single, married, partnered, separated or divorced and those who had completed the elementary school had significantly lower score than the participants of the rest educational categories. Similarly, other researchers have found that health literacy is lower in individuals with lower education [4-5].

As concerns the MedDietScore, those who were patients and had normal weight had significantly higher score than those who weren't patients and were obese. These results indicate that hypothesis 3 was also partially confirmed.

The age of the participants was negatively correlated with the health literacy. This means that the older the participant, the lower his/her health literacy. This result is consistent with that of other studies, in which health literacy was found to be lower in older ages [4-5]. However, the age was not associated with the adherence to the Mediterranean diet.

On the contrary, the BMI of the participants was significantly correlated only with the adherence to the Mediterranean diet and not with the health literacy. That is, the higher the participants' BMI, the higher the adherence to the Mediterranean diet. This result seems unexpected. However, this correlation was very weak. These results indicate that hypotheses 4 and 5 were only partially confirmed.

With regard to the use of the Internet for finding health information, women used it more frequently than men. This result is consistent with that found by Bujnowska-Fedak in the Polish general population [9].

Furthermore, those who weren't patients and had children used the Internet more frequently than patients and those who hadn't children for this reason. Diaz et al. [8] found in their study that about one out of two (53.5%) of the patients used the Internet for medical information. However, possible differences between patients and not patients have not been extensively evaluated. Similarly, studies have not examined possible differences between those who have and haven't children. Consequently, the above findings are preliminary results, which need further examination.

The main advantage of this study is that it covers a large gap in the scientific field and gives evidence that can be used by health practitioners. Thus, the strengths of this research include its originality for the Greek general population. Furthermore, the advantages of the Mediterranean diet in modern times demonstrate its importance in everyday clinical practice.

A primary limitation is the type of this study (cross-sectional), in which causal relationships cannot be supported. Moreover, the self-selection sampling method possibly introduces bias and reduces the representativeness of the general population. Due to the self-selection of our sample, it is likely that people who participated in this study have high level of health literacy and this could have skewed the results.

Future research is suggested to further investigate and clarify the results of this study. It would be beneficial to

conduct a longitudinal survey to overcome the limitations of the cross-sectional studies. Moreover, similar research could be carried out with a larger sample and comparison between healthy people and patients, in order to find possible differences. Future studies could also focus on people with particular characteristics and needs, e.g., patients with high comorbidity, with diseases who need a high adherence to the Mediterranean dier (eg. cardiovascular diseases) etc.

CONCLUSIONS

In this study, health literacy wasn't associated with the adherence to the Mediterranean diet and several demographic and medical characteristics impacted the health literature and the adherence to the Mediterranean diet. In addition, the majority of our sample had sufficient level of health literacy and medium adherence to the Mediterranean diet.

It is, therefore, necessary to recognize those who present low adherence to the Mediterranean diet and to implement interventions for their support. It is also important to improve people's health literacy (especially in older people), so that they can make better decisions about their lifestyle and healthy diet. In this way, they can avoid diseases associated with a sedentary lifestyle, reducing the prevalence of many non-communicable chronic diseases. The application of health literacy and adherence to Mediterranean diet in the health promotion programs and in the primary health care may also be valuable, and its evaluation could be a main component of their care.

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ORIGINAL ARTICLE

PREVALENCE OF HEALTHCARE-ASSOCIATED CERVICITIS AND ANTIMICROBIAL RESISTANCE OF THE RESPONSIBLE PATHOGENS IN UKRAINE: RESULTS OF A MULTICENTER STUDY (2019-2021)

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ABSTRACT

The aim: To obtain the first estimates of the current prevalence of healthcare-associated cervicitis (HACs) and antimicrobial resistance of responsible pathogens in Ukraine.

Materials and methods: We conducted a retrospective multicentre cohort study was based on surveillance data from January 1st, 2019 to December 31st, 2021 in Ukraine. Antibiotic susceptibility testing was determined by Kirby–Bauer disc diffusion test according to the protocol of the European Committee on Antimicrobial Susceptibility Testing.

Results: Of the 6,885 participants in this study, 1746 women (25.5%) met the clinical definition of cervicitis. Prevalence of HACs and cervcits caused sexually transmitted pathogens were 12.7% and 8.3%, respectively. The incidence of HACs among women with a history of gynecological procedures was 25.4%. The main causes of HACs were legal induced abortions (28.8%), vaginal hysterectomy (23.9%), and postpartum instrumental examination (12.8%). The predominant pathogens of HACs were: *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*. Methicillin-resistance was observed in 20.8% of *S. aureus* (MRSA). Vancomycin resistance was observed in 7.4% of isolated enterococci (VRE). Resistance to third-generation cephalosporins was observed in 13.1% *Klebsiella* spp. and *E.coli* 17.5% isolates. Carbapenem resistance was identified in 11.6% of *P.aeruginosa* isolates. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in K. pneumoniae (33.5%, vs 8.7%). The overall proportion of extended spectrum beta-lactamases (ESBL) production among *Enterobacteriaceae* was 34.6%.

Conclusions: This study showed that the prevalence of healthcare-associated cervicitis in Ukraine is high, and many cases were caused by antibiotic-resistant pathogens.

KEY WORDS: pelvic inflammatory disease, healthcare-associated cervicitis, prevalence, risk factors, antimicrobial resistance, Ukraine

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INTRODUCTION

Reproductive tract infections in female are common worldwide and represent a major public health problem with high morbidity, mortality and cost implications [1-4]. One of these diseases is a cervicitis. Cervicitis is the inflammation of the cervix. Inflammation is localised mainly in the columnar epithelial cells of the endocervical glands, but it can also affect the squamous epithelium of the ectocervix [5]. Despite the fact that there are not many studies on cervicitis, it is estimated that it is a common condition, with prevalence's as high as 20–40% in women [6].

A significant association has been found between cervicitis and the risk of affect the upper genital tract, such as endometritis and pelvic inflammatory disease (PID), infertility and adverse effects in pregnancy and birth, including ectopic pregnancy, premature rupture of membranes, spontaneous abortion, premature birth, and childhood morbidity [7-10]. Approximately 40% of cases of spontaneous preterm birth (sPTB) are associated with ascending intrauterine infections [11]. Previous studies have established bacterial (i.e., chlamydia, gonorrhea, mycoplasma, etc.) and viral infections (i.e., herpesviruses and human papillomaviruses) as risk factors of PTB [11]. However, the exact mechanism leading to PTB is still unknown. It is also reported that chronic inflammation of the cervix could contribute to the development of cervical cancer [12], In addition, cervicitis is thought to play an important role in the transmission of HIV infection, by increasing susceptibility to HIV infection and increased HIV viral shedding [13].

Cervicitis is a frequently asymptomatic, inflammatory condition of the cervix. It is common with rates as high as 30–45% in some clinic populations [6, 8]. Cervicitis can persist or recur after completing one or several cycles of antibiotic treatment [14]. This raises concerns about the appropriateness of empirical treatments currently

used to treat women with cervicitis. A lack of consensus on definitive treatments for cervicitis treatment guidelines reflects these uncertainties, with the potential for over-use of antibiotics. The clinical significance of the finding of cervicitis, especially in asymptomatic, has been debated.

There are multiple agents, as infectious and non-infectious, potentially involved in cervicitis. Most research focuses on infectious agents that are sexually transmitted and other potentially involved genital tract microorganisms [5]. According to literature, organisms variably implicated in the pathogenesis of cervicitis include *Chlamydia trachomatis, Neisseria gonorrhoeae*, *Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum*, *Ureaplasma urealyticum*, bacterial vaginosis, *Herpes simplex virus* (HSV), *Cytomegalovirus* (CMV), and adenovirus [5, 15-17]. *Chlamydia* and *N.gonorrhoea* account for less than half of cervicitis cases, with a largely undefined aetiology in the remainder, referred to as nonchlamydial, nongonococcal cervicitis or nonspecific cervicitis. The etiology in a high percentage of women in whom none of the known pathogens are found is still unknown. At the same time, the role of opportunistic gram-positive and gram-negative organisms, which are the main pathogens of reproductive tract infections in female, in the pathogenesis of cervicitis has not been sufficiently studied.

The prevalence of cervicitis associated with gynecological surgery is still not well known, in part due to the lack of surveillance for these infections. The importance of detection cervicitis and correct treatment lies in the fact that silent infection can result in complications such as salpingitis, endometritis and PID and have severe consequences in pregnant women. However, wide variations of case definition, study populations and methods for pathogen isolation hinder the ability to draw conclusions on the aetiology, natural history and best management of cervicitis on a population basis [18]. Most prevalence studies have been carried out in risk populations, mainly in patients from sexually transmitted infections clinics, which imply a bias and limits the conclusions with respect to the frequency of cervicitis associated with gynecological surgery or other medical procedures in the general population. Similar studies have not been conducted in Ukraine.

THE AIM

The aim of this study was to obtain the first estimates of the current prevalence of healthcare-associated cervicitis and antimicrobial resistance of responsible pathogens in Ukraine.

MATERIALS AND METHODS

DESIGN AND STUDY POPULATION

We conducted a retrospective multicentre cohort study was based on surveillance data for reproductive tract infection in female from January 1st, 2019 to December 31st, 2021 in Ukraine. The study population comprised women 17–45 years of age who were seeking health care at 10 women's health clinics (located in Odessa, Ivano-Frankivsk, Vinnytsia, Volyn, Rivne, Chernivtsi, Lviv, Poltava, Cherkasy, Zhytomyr, Chernihiv, Dnipropetrovsk, and Kyiv, Ukraine) and a 6 clinics for the management of sexually transmitted diseases (located in Kyiv and Odessa, Ukraine). From each clinic, we studied a group of women referred for suspected mucopurulent cervicitis and a representative sample of other women attending the clinic. All participants in this study were local residents. Criteria for exclusion from the study included pregnancy, current menses, concomitant vulvovaginal candidiasis, use of intravaginal or systemic antimicrobials during the week before enrollment, and the presence of an intrauterine device.

DEFINITIONS

In this study healthcare-associated cervicitis are infections in women get while they are receiving health care for another reproductive tract condition. Cervicitis was defined as the presence of either mucopurulent (yellow) endocervical discharge and/or endocervical bleeding (graded as "moderate" or "severe," as defined by the clinician) induced by gentle passage of a cotton swab. In addition, cervical infection must occur 48 hours after gynecological procedures. Cervicitis can happen in any health care facility, including hospitals and ambulatory centers. From the clinical point of view, cervicitis among women was classified as acute or chronic. In our study 'Thin' means that Body Mass Index (BMI) is less than 18.5 kg/m^2. 'Normal' means 18.5 kg/m2≤ BMI < 24 kg/m^2. 'Overweight' means 24 kg/m^2≤ BMI < 28 kg/m^2. 'Obesity' means that BMI is more than 28 kg/m^2.

Table I. Distribution of healthcare-associated cervicitis by type of gynecological procedures in Ukraine (2019-2021)

Characteristics of procedure	Number of women (n)	Healthcare-associated cervicitis				95% CI
		No		Yes		
		n	%	n	%	
Legal induced abortions	2043	1455	71,2	588	28,8	28.0 – 29.6
Vaginal hysterectomy	961	732	76,1	229	23,9	23.2 – 24.6
Postpartum instrumental examination	438	382	87,2	56	12,8	12.2 – 13.4
Total	3442	2569	74,6	873	25,4	24.7 – 26.1

CI. Confidence interval

Table II. Characteristics of women with healthcare-associated cervicitis in Ukraine (2019-2021)

Characteristucs	All (n=3,442)	Healthcare-associated cervicitis				p-value
		No		Yes		
		n	%	n	%	
Age (years)						
17-21	1,.178	814	31.7	364	41.7	< 0.001
22-26	670	427	16.6	243	27.8	
27-31	428	320	12.5	108	12.4	
32-36	810	711	27.7	99	11.3	
37-41	197	148	5.8	49	5.6	
42-45	159	149	5.8	10	1.1	
Place of residence						
Urban	1,804	1,357	52.8	447	51.2	0.523
Rural	1,638	1,212	47.2	426	48.8	
Occupation						
Unemployed	468	333	13.0	135	15.5	0.517
Head of enterprises	486	356	13.9	130	14.9	
Professional worker	1,264	967	37.6	297	34.0	
Clerk	131	108	4.2	23	2.6	
Service worker	472	355	13.8	117	13.4	
Agricultural and related worker	90	63	2.5	27	3.1	
Operator	45	36	1.4	9	1.0	
Other	486	351	13.7	135	15.5	
Education						
Primary	486	338	13.2	148	17.0	0.374
High school	522	409	15.9	113	12.9	
Junior college degree	756	575	22.4	181	20.7	
Bachelor's degree and above	1,678	1,247	48.5	431	49.4	
Smoking						
No	45	36	1.4	9	1.0	0.564
No, secondhand smoke	801	598	23.3	203	23.2	
Yes	2,596	1,935	75.3	661	75.7	
Drinking						
No	400	333	13.0	67	7.7	0.072
Yes	3,042	2,236	87.0	806	92.3	
BMI (kg/m^2)						
Thin	248	176	6.9	72	8.2	0.454
Normal	2,173	1,651	64.3	522	59.8	
Overweight	778	562	21.9	216	24.7	
Obese	243	180	7.0	63	7.2	
Irregular menstruation						
No	2,362	1,786	69.5	576	66.0	0.218
Yes	1,080	783	30.5	297	34.0	
Married						
No	1,948	1,458	56.8	490	56.1	0.561
Yes	1,494	1,111	43.2	383	43.9	
Unmarried						

Table II. Characteristics of women with healthcare-associated cervicitis in Ukraine (2019-2021) (cont.)

No	1,714	1,358	52.9	356	40.8	0.047
Yes	1,728	1,211	47.1	517	59.2	
History of PID						
No	3,091	2,394	93.2	697	79.8	< 0.001
Yes	351	175	6.8	176	20.2	
The presence of an intrauterine device						
No	1,300	913	35.5	387	44.3	0.009
Yes	2,142	1,656	64.5	486	55.7	
History of endometriosis						
No	3,348	2,515	97.9	833	95.4	0.041
Yes	94	54	2.1	40	4.6	
Bacterial vaginosis	3,442	2,569		873		
No	3,262	2,497	97.2	765	87.6	< 0.001
Yes	180	72	2.8	108	12.4	
Total	3,442	2,569	74.6	873	25.4	

BMI, Body Mass Index
PID, Pelvic Inflammatory Disease

Table III. Logistic multivariate regression analyses of risk factors for healthcare-associated cervicitis in Ukraine (2019-2021)

Characteristics	*p*-value	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)
Age (years)				
	< 0.001		< 0.001	
17–21		Ref		Ref
22-26	0.003	9.379 (2.165–40.619)	0.011	6.862 (1.557–30.247)
27–31	0.012	6.618(1.549–28.274)	0.031	5.036 (1.163–21.83)
32–36	0.025	5.577 (1.244–25.011)	0.109	3.49 (0.758–16.071)
37–41	0.035	5.50 (1.131–26.752)	0.174	3.096 (0.607–15.797)
42–45	0.269	2.297(0.515–10.249)	0.587	1.523 (0.335–6.943)
History of endometriosis				
No		Ref		Ref
Yes	0.041	5.53 (1.136–26.781)	0.173	3.093 (0.608–15.792)
The presence of an intrauterine device				
No		Ref		Ref
Yes	0.009	3.623 (2.231–5.841)	< 0.001	3.081 (1.816–5.157)
History of PID				
No		Ref		Ref
Yes	< 0.001	3.611 (2.234–5.831)	< 0.001	3.063 (1.819–5.158)
Bacterial vaginosis				
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

OR, Odd Ratio
PID, Pelvic Inflammatory Disease

DATA COLLECTION

This study includes interviews and questionnaires of women's, also analyses medical records. We formed a working group to conduce this research, which included obygyne specialists (nurses and physicians). After written, informed consent was obtained from each woman, the women were interviewed

Table IV. Antibiotic susceptibility of gram-positive bacteria isolated from women with healthcare-associated cervicitis in Ukraine (2019-2021)

Antibiotic	*S. aureus* (n=103)		*S.epidermidis* (n=52)		*Streptococcus* spp. (n=39)		*E. faecalis* (n=80)	
	S	R	S	R	S	R	S	R
PEN	28.3	71.7	30.7	69.3	35.2	64.8	72.9	27.1
AMP	81.7	18.3	89.2	10.8	48.3	51.7	88.1	11.9
SAM	88.9	17.1	78.6	21.4	94.1	5.9	38.8	61.2
OXA	79.2	20.8	81.3	18.7	NT	NT	NT	NT
CXM	68.8	31.2	81.5	18.5	86.2	13.8	NT	NT
CRO	81.5	18.5	86.2	13.8	94.1	5.9	NT	NT
EPM	NT	NT	NT	NT	NT	NT	100.0	0
GEN	87.4	12.6	91.5	8.5	91.2	8.8	81.1	18.8
AMK	92.4	7.6	100.0	0	95.9	4.1	76.8	23.2
CLI	83.5	16.5	78.8	21.2	89.8	16.2	11.9	88.1
AZM	65.9	34.1	71.6	28.4	68.8	31.2	84.9	15.1
VAN	100.0	0	100.0	0	NT	NT	92.6	7.4
CIP	78.3	21.7	NT	NT	NT	NT	91.1	8.9
LVX	83.7	16.3	74.2	25.8	87.2	12.8	92.8	7.2
LNZ	100.0	0	100.0	0	99.8	0.2	100.0	0

R, resistant isolates (%); S, susceptible isolates (%); NT, no tested; NT, no tested;
PEN, penicillin; AMP, ampicillin; SAM, ampicillin/sulbactam; OXA, oxacillin; CXM, cefuroxime; CRO, ceftriaxone; EPM, ertapenem; GEN, gentamicin; AMK, amikacin; CLI, clindamycin; AZM, azithromycin; VAN, vancomycin; CIP, ciprofloxacin; LVX, levofloxacin; LNZ, linezolid.

about their medical history. Women were approached face-to-face in medical clinics 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, medical history, and personal lifestyle habits, under the guidance of this specialist. In our study adopted double entry mode of paper questionnaire data and were analysed anonymously. In addition, all patients underwent a standardized physical examination, including speculum examination and visual inspection and collection of endocervical fluid for cultural microbiological analysis. Current douching and smoking were defined as douching and smoking that occurred during the month before enrollment, and sexual practices were assessed by asking subjects to estimate the recent frequency of and the time to last occurrence of specific behaviors.

MICROBIAL ANALYSIS

Two endocervical and two vaginal swabs were collected and processed. Endocervical swab specimens were collected and used for aerobic and anaerobic cultures, as well as for antibiotic susceptibility testing. Swab specimens obtained for culture were immediately placed into Port-a-Cul anaerobic transport tubes (Becton Dickinson), which were held and transported at room temperature to the laboratory and were prepared for culture immediately on receipt. In this study species identification was performed with standard microbial methods. In this study bacterial species identification was performed with standard microbial methods. Antibiotic susceptibility testing of bacteria was determined by Kirby–Bauer disc diffusion test according to the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://eucast.org). Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) were defined by resistance to oxacillin and vancomycin respectively. Multidrug resistance (MDR) for *Acinetobacter* spp. and *Pseudomonas aeruginosa* was defined in accordance with published definitions, which were used in NHSN AMR report [19]. An isolate of *Acinetobacter* spp was defined as having MDR if it tested nonsusceptible to at least 1 drug in 3 of the following 6 antimicrobial agents/groups: piperacillin or piperacillin/tazobactam, extended-spectrum cephalosporins (cefepime or ceftazidime), aminoglycosides, ampicillin/sulbactam, carbapenems, and fluoroquinolones. For *P. aeruginosa* isolates, MDR was defined as testing nonsusceptible (ie, either resistant or intermediate) to at least 1 drug in 3 of the 5 following antimicrobial groups: piperacillin or piperacillin/tazobactam, extended-spectrum cephalosporins (cefepime or ceftazidime), fluoroquinolones, aminoglycosides, and carbapenems. Multi-drug resistance for *Klebsiella pneumoniae* and *Escherichia coli* was defined as being non susceptible to at least 1 drug in 3 antimicrobial agents/groups [20].

ETHICS

Ethical clearance for this study was obtained from the ethics committee of the Shupyk National Healthcare University of Ukraine. This study was performed in line with the principles

Table V. Antibiotic susceptibility of gram-negative bacteria isolated from women with healthcare-associated cervicitis in Ukraine (2019-2021)

Antibiotic	*E. coli* (n=548)		*Enterobacter* spp. (n=129)		*Klebsiella* spp. (n=105)		*Proteus* spp. (n=55)		*P. aeruginosa* (n=84)	
	S	R	S	R	S	R	S	R	S	R
AMX	65.2	34.8	NT	NT	NT	NT	70.4	29.6	NT	NT
AMC	78.1	21.9	39.8	60.2	85.2	14.8	84.3	15.7	NT	NT
TIC	69.9	30.1	92.7	7.3	NT	NT	86.5	13.5	81.9	18.1
TZP	96.3	3.7	96.5	3.5	100.0	0	100.0	0	77.2	22.8
CXM	63.8	36.2	77.4	22.6	87.6	12.4	NT	NT	NT	NT
CTX	87.1	12.9	96.1	3.9	88.3	11.7	98.8	1.2	NT	NT
CRO	72.2	27.8	65.9	34.1	73.9	26.1	NT	NT	NT	NT
CAZ	91.4	8.6	96.2	3.8	92.1	7.9	94.5	5.5	87.8	12.2
FEP	93.3	6.7	100.0	0	77.6	22.4	96.7	3.3	51.2	48.8
IPM	87.1	12.9	100.0	0	91.3	8.7	98.3	1.7	84.6	15.4
MEM	NT	NT	NT	NT	NT	NT	NT	NT	91.3	8.7
EPM	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0
GEN	94.5	5.5	91,2	8.8	91.2	8.8	98.8	1.2	63.8	36.2
AMK	89.4	10.6	92.7	7.3	82.6	17.4	100.0	0	84.7	15.3
CIP	87.2	12.8	98.6	1.4	95.1	4.9	75.1	24.9	81.2	18.8
LVX	67.3	32.7	78.7	21.3	92.7	7.3	NT	NT	NT	NT
CFP	NT	NT	NT	NT	NT	NT	NT	NT	66.2	33.8

R, resistant isolates (%); S, susceptible isolates (%); NT, no tested; NT, no tested;
AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; TIC, ticarcillin; TZP, piperacillin/tazobactam; CXM, cefuroxime; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; EPM, ertapenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; LVX, levofloxacin; CFP, cefoperazone.

of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study. Participants' data were anonymised prior to the analysis, and each had a unique identification number assigned to protect their privacy.

STATISTICAL ANALYSIS

In this study all clinical and microbiological data were entered in an Excel (Microsoft Corp., Redmond, WA, USA) database for statistical analysis. Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. Proportions of total cervicitis cases meeting specific criteria were calculated, and characteristics of each category were compared by using Fisher exact test. Multivariable analysis was done including all the potential risk factors. Standard techniques for continuous variables and an unadjusted chi-square test for binary variables were used for the univariate analysis. All statistical analyses were 2-sided and significance was set at $p<0.05$.

RESULTS

PREVALENCE OF CERVICITIS

Between January 2019 and December 2021, 6852 women participated in the study. Of these, 1746 women (25.5%) met the clinical definition of cervicitis. Prevalence of healthcare-associated cervicitis and cervcits caused sexually transmitted pathogens were 12.7% [95% confidence interval (CI), 12.3-13.1] and 8.3% (95% CI, 8-8.6%), respectively. The etiology of 4.4% (301/6852) cases cervicitis was unknown. The incidence of healthcare-associated cervicitis among women with a history of gynecological procedures was 25.4% (95% CI, 24.7-26.1%). From data obtained, the main causes of healthcare-associated cervicitis were legal induced abortions (28.8%, 95% CI, 28.0-29.6), vaginal hysterectomy (23.9%, 95% CI, 23.2-24.6), and postpartum instrumental examination (12.8%, 95% CI, 12.2-13.4). The characteristics of these procedures are presented in Table I.

PATIENT CHARACTERISTICS AND RISK FACTORS

In this study the difference in the age, history of endometriosis, the presence of an intrauterine device, history of PID, and bacterial vaginosis between the two groups were statistically significant ($p < 0.05$). The healthcare-associated cervicitis rate rose initially and then decreased for every 4 years added to women's age. Women aged 17-21 years and 22-26 years had the highest healthcare-associated cervicitis rate. When the participants our study were grouped by age the healthcare-associated cervicitis rate increased as the age. It was found that among those women who had healthcare-associated cervicitis , more were from rural than

urban. Among healthcare-associated cervicitis women 67.3% went to clinc seeking medical help regardless of the outcome after treatment. Characteristics of women with healthcare-associated cervicitis and risk factors are presented in Table II.

In this study Table III showed the odds ratio (OR) and 95% confidence interval (CI) for the risk factors associated with cervicitis in logistic multivariate regression analyses. Healthcare-associated cervicitis was associated with age as shown in logistic regression analysis. Also, history of PID, bacterial vaginosis, the presence of an intrauterine device, and history of endometriosis was associated with healthcare-associated cervicitis. There were differences among factors associated with cervicitis. In our study the risk factors associated with cervicitis were age of women (< 0.001), history of PID (< 0.001), the presence of an intrauterine device (p= 0.009), bacterial vaginosis (< 0.001), and history of endometriosis (p=0.041).

RESPONSIBLE PATHOGENS AND ANTIMICROBIAL RESISTANCE

In this study a total of 1208 different bacterial strains were isolated from 873 women's with healthcare-associated cervicitis. Aerobic Gram-negative bacilli make up 77.5% and 22.5% Gram-positive cocci from of all isolates. The predominant pathogens were: *Escherichia coli* (45.4%), *Enterobacter* spp. (10.7%), *Klebsiella* spp. (8.7%), *Staphylococcus aureus* (8.5%), *Pseudomonas aeruginosa* (7%), *Enterococcus faecalis* (6.6%), *Proteus* spp. (4.6%), *Staphylococcus epidermidis* (4.3%), followed by *Streptococcus* spp. (3.2%) and *Acenetobacter* spp. (1.1%).

Antimicrobial susceptibility tests were performed on a total of 274 isolates of Gram-positive cocci and 934 Gram-negative organisms. In present study the antimicrobials used in antimicrobial susceptibility testing included those commonly used as therapeutic agents. Varying degrees of resistance to most antibiotics tested were found. The antibiotic susceptibility profiles of isolates from women's with healthcare-associated cervicitis are presented in Table IV and Table V.

In this study the overall proportion of methicillin-resistance was observed in 20.8% of *S. aureus* (MRSA). Vancomycin resistance was observed in 7.4% of isolated enterococci (VRE). Resistance to third-generation cephalosporins was observed in 13.1% *Klebsiella* spp. and *E.coli* 17.5% isolates. Carbapenem resistance was identified in 11.6% of *P.aeruginosa* isolates. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (33.5%, vs 8.7%). The overall proportion of extended spectrum beta-lactamases (ESBL) production among *Enterobacteriaceae* was 34.6%.

DISCUSSION

This study presents the first estimates data on prevalence of healthcare-associated cervicitis and antimicrobial resistance of responsible pathogens in Ukraine. I during study period was identified a high level of cervicitis (25.5%) in women. I during study period was identified a high level of cervicitis (25.5%) in women. Prevalence of healthcare-associated cervicitis and cervcits caused sexually transmitted pathogens were 12.7% and 8.3%, respectively. The etiology of 4.4% cases cervicitis was unknown. The incidence of healthcare-associated cervicitis among women with a history of gynecological procedures was 25.4%. The main causes of healthcare-associated cervicitis were legal induced abortions (28.8%), vaginal hysterectomy (23.9%), and postpartum instrumental examination (12.8%). In our study age of women (< 0.001), history of PID (< 0.001), the presence of an intrauterine device (p= 0.009), bacterial vaginosis (< 0.001), and history of endometriosis (p=0.041) were associated with a higher risk of contracting a healthcare-associated cervicitis. The risk factors identified in this study are consistent with previous studies [4].

The prevalence of healthcare-associated reproductive tract infection in women varies from country to country and ranges from 1.8% [21] to 48% [22]. However, the available literature does not contain data on the prevalence of healthcare-associated cervicitis in Ukraine and other countries. Therefore, we were unable to compare our results with other studies in other countries.

In this study the predominant pathogens of healthcare-associated cervicitis were: *E. coli, Enterobacter* spp., *Klebsiella* spp., *S. aureus, P. aeruginosa*, *E. faecalis*, *Proteus* spp. *S. epidermidis,* followed by *Streptococcus* spp. and *Acenetobacter* spp. Our results was coherent with reports other studies that focus on female healthcare-associated PID [4, 22-24].

In the past several years, the generally understanding of cervicitis has extended beyond the recognition of *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, HSV, *M.genitalium*, and bacterial vaginosis as the prime etiologic suspects. However, major gaps in our knowledge of this common condition remain. Putative etiologic agents have not been identified in many women with cervicitis. Moreover, cervicitis occurs in a relatively small proportion of women with chlamydia or gonorrhea [6]. Whereas *Chlamydia*, *N. gonorrhoea* and syphilis have been extensively examined, there remains a paucity of knowledge of healthcare-associated cervicitis, an arguably more prevalent but poorly characterized condition with uncertain clinical implications. In addition, scant research has addressed the clinical response of nonchlamydial and nongonococcal cervicitis to antibiotic therapy.

Increasing broad-spectrum antibiotic usage with associated emergence of antimicrobial resistance reinforces the need for targeted antibiotic therapies, including the management of cervicitis. However, a lack of consensus on definitive treatments for cervicitis treatment guidelines reflects uncertainties, with the potential for over-use of antibiotics. This raises concerns about the appropriateness of empirical treatments currently used to treat women with cervicitis.

In our study the overall proportion of methicillin-resistance was observed in 20.8% of *S. aureus* (MRSA). Vancomycin

resistance was observed in 7.4% of isolated enterococci (VRE). Resistance to third-generation cephalosporins was observed in 13.1% *Klebsiella* spp. and *E.coli* 17.5% isolates. Carbapenem resistance was identified in 11.6% of *P.aeruginosa* isolates. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (33.5%, vs 8.7%). The overall proportion of extended spectrum beta-lactamases (ESBL) production among *Enterobacteriaceae* was 34.6%.

Current international and most national guidelines for the management of pelvic inflammatory disease recommend the prescription of antibiotics for prophylactic and treatment [22, 25]. However, the appointment of an inadequate starting therapy decreases the effectiveness of treatment infection. Microbiological monitoring of the antimicrobial resistance of the responsible pathogens of healthcare associated cervicitis is necessary to enhance our knowledge of epidemiology. Therefore, this was the basis for this study.

Advances in surveillance will facilitate this process, but antibiotic susceptibility testing of responsible pathogenes should not replace clinical examination while cervicitis prevalence and significance is not yet established. A standardized approach to healthcare-associated cervicitis research, particularly with consensus of case definition, may facilitate outcomes that can be more generally applied in surveillance and clinical practice.

STRENGTHS AND LIMITATION

Strengths: This was a first multi-center study based on surveillance data and designed to evaluate the prevalence current prevalence of healthcare-associated cervicitis and antimicrobial resistance of responsible pathogens in Ukraine.

Limitation: The retrospective nature of this study. Despite this, important prevalence healthcare-associated cervicitis data were obtained that can be used for comparison between countries. In addition, these data will be useful for improving infection control activities and developing a new strategy for the control and prevention of HAIs.

CONCLUSIONS

This study showed that the prevalence of healthcare-associated cervicitis in Ukraine is high, and many cases were caused by antibiotic-resistant pathogens. As our understanding of the aetiology and significance of cervicitis, particularly healthcare-associated cervicitis, improves, management will be refined. New research into the etiology and antibiotic resistance of responsible pathogens, and natural history of this common condition is needed, especially in view of the well-established links between cervicitis and an increased risk of upper genital tract infection. To prevent the healthcare-associated cervicitis it is necessary to develop and implement advanced infection control measures that are based on surveillance data.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

MORPHOLOGICAL AND FUNCTIONAL FEATURES OF THE MUCOUS MEMBRANE OF SMALL AND LARGE INTESTINE IN PATIENTS WITH COVID-19 AND IN POST-COVID-19 PERIOD

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ABSTRACT

The aim: To reveal the morphological and functional features of the mucous membrane of small and large intestine in patients with COVID-19 and in post-COVID-19 period.

Materials and methods: In the present study, the authors used biopsy and autopsy material represented by the fragments of the mucous membrane of small and large intestine. All studied material was divided into 10 groups. Group 1 (comparison group) included autopsy material from the deceased who did not have COVID-19 during their lifetime. Groups 2-4 included autopsy material from the deceased who had COVID-19 of varying severity during their lifetime. Groups 5-7 included biopsy material from patients who had recovered from COVID-19 of varying severity, while the duration of the post-COVID period ranged from 1 to 50 days. Groups 8-10 included biopsy material from patients who had in anamnesis COVID-19 of varying severity (the duration of the post-COVID period lasted from 51 to 100 days). Histological, immunohistochemical, morphometric and statistical research methods were used.

Results: The comparative analysis showed a more expressed deficiency of ACE2 in the mucous membrane of small and large intestine in patients with moderate and severe COVID-19 compared with patients in post-COVID-19 period of different duration. In patients who had moderate and severe COVID-19 in anamnesis, ACE2 deficiency decreases with increasing duration of post-COVID-19 period. In patients recovered from mild COVID-19, the ACE2 content increases with the duration of post-COVID-19 period from 1 to 50 days and corresponds to the norm with the duration of this period from 51 to 100 days.

Conclusions: The comprehensive morphological study conducted by the authors made it possible, firstly, to clarify the morphological and functional features of the mucous membrane of small and large intestine in patients with COVID-19 of various degrees of severity; secondly, to obtain new data about the morpho-functional state of the mucous membrane of small and large intestine in patients, taking into account different duration of the post-COVID-19 period and the severity of the infection.

KEY WORDS: morphological and functional features, mucous membrane, small and large intestine, COVID-19, post-COVID-19 period

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmission infection disease which has made a global impact due to its ability to spread rapidly, and its relatively high morbidity and mortality rate [1]. Along with the respiratory system, the gastrointestinal tract is one of the main extra-pulmonary targets of SARS-CoV-2 [2]. Numerous gastrointestinal tract symptoms (anorexia, diarrhea, nausea/vomiting, abdominal pain/discomfort и т.д.) have been reported in the literature in patients with COVID-19, with the incidence of these symptoms ranging widely from 2% to 79.1% [3, 4]. Mechanisms of gastrointestinal tract damage in COVID-19 are still questionable. Some scientists have explained the COVID-19-related gastrointestinal tract injury by drug-induced injury, systemic inflammatory reaction, hypoxia–ischemia reperfusion injury. However, according to many scientists, a key link in the mechanism of damage of the gastrointestinal tract in COVID-19 is presence of angiotensin-converting enzyme 2 (ACE2) in mucosa, due to which the virus enters the host cell [5]. The downregulation of ACE2 leads to gastrointestinal dysfunction [6].

Gastrointestinal dysfunction is often diagnosed in patients in post-COVID-19 period [7]. Such patients experienced loss of appetite, nausea, weight loss, abdominal pain, heartburn, dysphagia, altered bowel motility and irritable bowel syndrome [8]. The mechanism of these violations

Table I. Division of the studied material into groups

Group number	Group characteristics	Number of cases
1	Autopsy material from the deceased who did not suffer from COVID-19 during their lifetime	10
2	Autopsy material from the deceased, who suffered from mild COVID-19 during their lifetime	12
3	Autopsy material from the deceased who suffered from moderate COVID-19 during their lifetime	11
4	Autopsy material from the deceased who suffered from severe COVID-19 during their lifetime	13
5	Biopsy material from patients who had mild COVID-19 (duration of the post-COVID period lasted from 1 to 50 days)	11
6	Biopsy material from patients who had moderate COVID-19 (the duration of the post-COVID period lasted from 1 to 50 days)	13
7	Biopsy material from patients who had severe COVID-19 (the duration of the post-COVID period lasted from 1 to 50 days)	12
8	Biopsy material from patients who had mild COVID-19 (the duration of the post-COVID period lasted from 51 to 100 days)	10
9	Biopsy material from patients who had moderate COVID-19 (the duration of the post-COVID period lasted from 51 to 100 days)	12
10	Biopsy material from patients who had severe COVID-19 (the duration of the post-COVID period lasted from 51 to 100 days)	9
	Total number of cases	113

development is not completely understood today. Some scientists suggest that gut microbiome dysbiosis may play a key role in the development of post-COVID-19 gastrointestinal tract symptoms [9].

Literature data about the morphological and functional features of the mucous membrane of small and large intestine in patients with COVID-19 are not systematized and do not take into account the severity of this infection. There is no information in the literature about the morphological features of the mucous membrane of small and large intestine in patients in post-COVID-19 period, taking into account the severity of the infection, as well as the duration of post-COVID-19 period itself. These facts dictate the need and emphasize the relevance of this study.

THE AIM

The purpose is to reveal the morphological and functional features of the mucous membrane of small and large intestine in patients with COVID-19 and in post-COVID-19 period.

MATERIALS AND METHODS

In the present study, the authors used biopsy and autopsy material represented by the fragments of the mucous membrane of small and large intestine. All studied material was divided into 10 groups. Group 1 (comparison group) included autopsy material from the deceased who did not have COVID-19 during their lifetime. Groups 2-4 included autopsy material from the deceased who had COVID-19 of varying severity during their lifetime. Groups 5-7 included biopsy material from patients who had recovered from COVID-19 of varying severity, while the duration of the post-COVID period ranged from 1 to 50 days. Groups 8-10 included biopsy material from patients who had in anamnesis COVID-19 of varying severity (the duration of the post-COVID period lasted from 51 to 100 days). Table I gives a detailed description of the formed groups.

Autopsy and biopsy material was fixed in a 10% solution of neutral buffered formalin according to the generally accepted technique and embedded in paraffin. Serial sections of 3-4 μm thick were made from paraffin blocks. The authors studied the slides stained with hematoxylin and eosin using an Olympus BX-41 microscope (Japan).

Immunohistochemical study was performed according to a standardized protocol using a monoclonal antibody (MCA) against ACE2 (anti-ACE2, clone 4G5.1, Sigma-Aldrich MABN59, replaces MAB5676; EMD Millipore Corporation, USA). The UltraVision Quanto HRP detection system (Thermo Fisher Scientific, USA) visualized primary antibodies. The immunohistochemical reaction was evaluated by applying the brightness factor in the Lab color model, using a computer program «Analysis of color properties of raster images» developed by Myroshnychenko M.S. et al. [10].

The obtained digital data were statistically processed, using the Statistica 10.0 program. The average indicators in the groups were compared, using the nonparametric Mann-Whitney U test. Differences were considered significant at $p<0.05$.

RESULTS

When studying the slides stained with hematoxylin and eosin in groups 2-4 in the mucous membrane of small and large intestine, the authors identified the same general pathological processes, the severity of which increased in

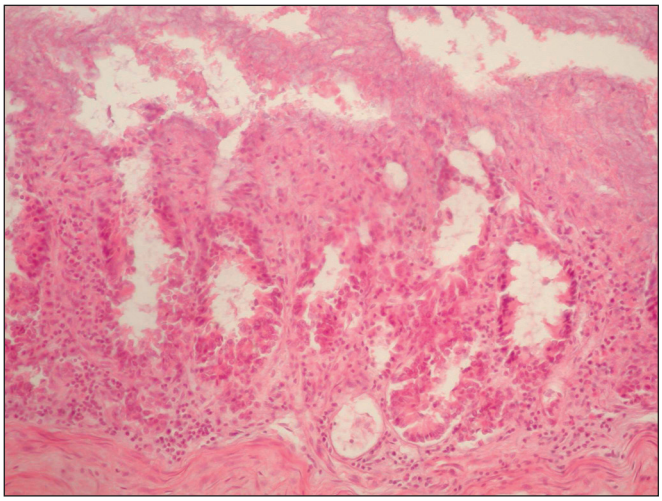

Fig. 1. Group 4. Diffuse extremely expressed alterative-desquamative changes of the epithelium, polymorphocellular inflammatory infiltration, hemodynamic disturbances in the mucous membrane of the colon. Stained with hematoxylin and eosin, ×200.

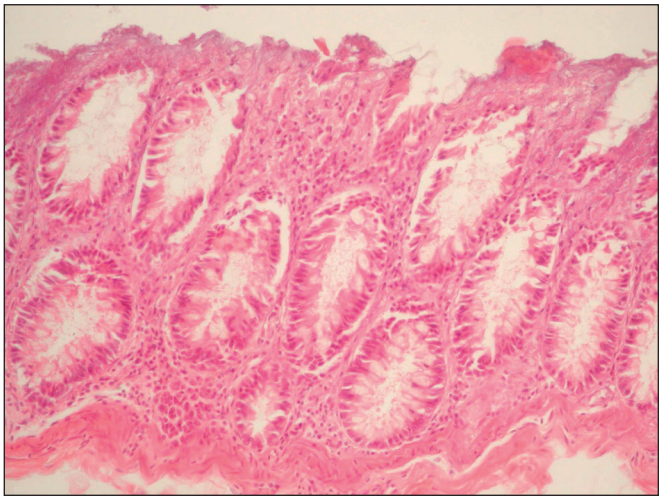

Fig. 2. Group 6. Diffuse moderately expressed alterative-desquamative changes in the surface epithelium and epithelium of the intestinal glands, diffuse moderately expressed polymorphocellular infiltration, moderately expressed hemodynamic disturbances in the mucous membrane of the colon. Stained with hematoxylin and eosin, ×200.

the direction from group 2 to group 4. These general pathological processes were characterized by diffuse dystrophic, necrotic and desquamative changes in the surface epithelium and epithelium of the intestinal glands; formation of acute erosions and ulcers; hemodynamic disturbances represented by edema, hemorrhages, vascular plethora, sludge phenomenon and thrombus formation; presence in the epithelial layer, proper and muscular layers of the mucous membrane of diffuse polymorphocellular infiltration, including lymphocytes, macrophages, plasma cells, mast cells, neutrophilic and eosinophilic leukocytes (Fig. 1).

In groups 5-7 we detected general pathological processes in the mucous membrane of small and large intestine of the same type, less expressed and similar to groups 2-4, the intensity of which increased in the direction from group 5

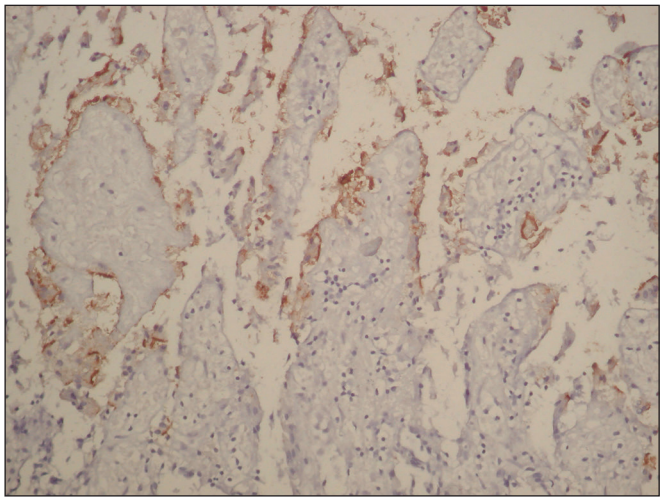

Fig. 3. Group 4. Expression of ACE2 by damaged and undamaged surface epitheliocytes of the small intestine mucosa. Immunohistochemical reaction with MCA against ACE2, ×200.

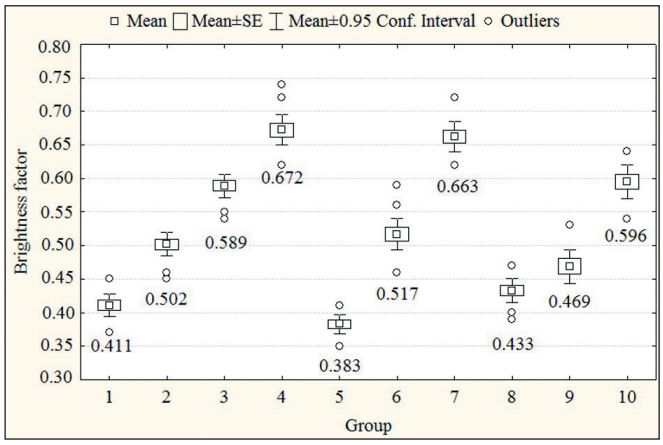

Fig. 4. Values of the brightness factor in small intestine in groups 1-10.

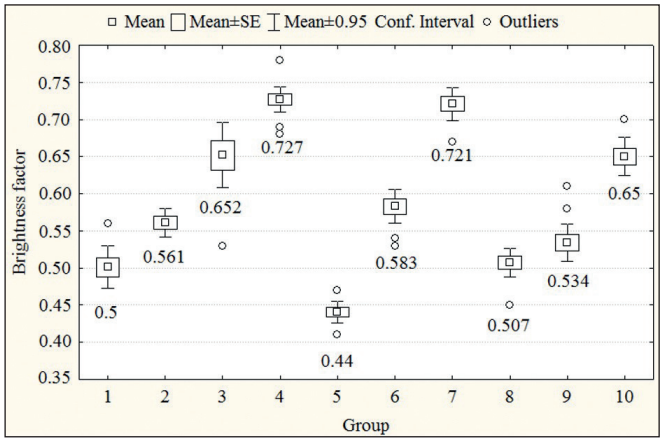

Fig. 5. Values of the brightness factor in large intestine in groups 1-10.

to group 7. Alterative-desquamative changes in the surface epithelium and epithelium of the intestinal glands were not determined in group 5, while in groups 6, 7 they were diffuse and, respectively, moderately and extremely expressed (Fig. 2). Erosive and ulcerative changes were found only in group 7. Signs of circulatory disorders, characterized by edematous changes, hemorrhages, vascular plethora,

sludge phenomenon and thrombus formation in the lumen of blood vessels in groups 5, 6 and 7 were, respectively, minimally, moderately and extremely expressed. Diffuse polymorphic cell infiltration was also detected in the mucous membrane which had similar qualitative characteristics to groups 2-4 and was, respectively, minimally, moderately, extremely expressed in groups 5, 6 and 7.

When studying the slides of groups 8-10, we noted that in group 8 the morphological picture of the mucous membrane of the small and large intestine corresponded to group 1. However, in groups 9 and 10, the same type of various general pathological processes was detected in the mucous membrane of small and large intestine.

The latter was less expressed compared with groups 6, 7, characterized by focal alterative-desquamative changes in the surface epithelium and epithelium of the intestinal glands in group 10; single acute erosions in group 10; minimally and moderately expressed hemodynamic disorders in groups 9 and 10, represented by edema, vascular congestion and sludge phenomenon. Diffuse polymorphic cellular infiltration in the mucosa in groups 9-10 was, respectively, minimally and moderately expressed and its composition was similar to groups 6-7.

In groups 1-10, positive expression of ACE2 in the form of brown staining was noted predominantly on the apical surface of the superficial epithelium of the small and large intestine mucosa (Fig. 3). The results of the brightness factor measurement are shown in Figures 4 and 5.

In a comparative analysis of the brightness factor in small and large intestine in groups 1-10, we noted a lower ($p<0.05$) value of this indicator in small intestine compared to the large intestine. This fact indicated a higher content of ACE2 in the small intestine compared to the large intestine.

In groups 2-4, the brightness factor in small and large intestine was greater ($p<0.05$) compared to group 1, indicating a decrease ($p<0.05$) in the content of ACE2 in the surface epithelium of the mucosa. The brightness factor in the direction from group 2 to group 4 increased ($p<0.05$), which also indicated a decrease in the content of this enzyme.

The brightness factor in group 5 decreased compared to group 1 ($p<0.05$), indicating an increase in the content of ACE2 in the mucous membrane of small and large intestine. However, in groups 6 and 7, the content of this enzyme decreased, as evidenced by an increase ($p<0.05$) in the value of this indicator. In the direction from group 5 to group 7, the brightness factor increased ($p<0.05$).

Comparing the brightness factor in groups 8-10 with group 1, there was no ($p>0.05$) difference in group 8 and its increase ($p<0.05$) in groups 9 and 10. In the direction from group 8 to group 10, the brightness factor increased ($p<0.05$).

The brightness factor in group 2 compared to groups 5 and 8, in group 3 compared to groups 6 and 9, in group 4 compared to groups 7 and 10 had a higher value ($p<0.05$). The brightness factor in group 5 compared to group 8 had a lower value ($p<0.05$), while in group 6 compared to group 9, in group 7 compared to group 10 was higher ($p<0.05$).

DISCUSSION

The functions of intestinal mucosa include digestion, absorption, excretion and protection [11]. Intestinal mucosa dysfunctions have been associated with a broad range of diseases [12].

During the survey microscopy of the slides in the mucous membrane of small and large intestine of patients with COVID-19, we revealed the same type of morphological changes. Their intensity grew with an increase in the infection severity. These changes were characterized by alteration and desquamation of the epithelial layer, erosive and ulcerative processes, hemodynamic disorders and presence of diffuse inflammatory polymorphocellular infiltration. The morphological changes in the mucosa of small and large intestine of patients with COVID-19, identified by the authors, coincide with the data of other scientists who interpret these changes as ischemic enterocolitis [13-15].

The morphological state of the mucous membrane of small and large intestine in patients in post-COVID-19 period, as shown by the study conducted by the authors, was of the same type and depended on the duration of this period.

Minimally expressed hemodynamic disturbances and diffuse polymorphocellular inflammatory infiltration were found in the mucous membrane of small and large intestine in cases where the duration of the post-COVID-19 period ranged from 1 to 50 days in patients who had mild COVID-19. In cases when patients suffered from COVID-19 of moderate severity, we discovered moderately expressed diffuse alterative-desquamative changes in the epithelial layer, signs of circulatory disorders and diffuse polymorphic cell infiltration in the mucosa. In cases of severe COVID-19, there were expressed alterative-desquamative changes in the epithelium, erosive and ulcerative processes, severe hemodynamic disturbances and diffuse polymorphocellular infiltration in the mucosa. A comparative analysis conducted by the authors showed that in this category of patients, the severity of general pathological processes in the mucous membrane of small and large intestine intensified with increasing severity of COVID-19 in anamnesis.

In cases when the duration of the post-COVID-19 period lasted from 51 to 100 days in persons who underwent mild COVID-19, the mucous membrane of small and large intestine completely restored and its morphological picture was normal. In cases when patients suffered from COVID-19 of moderate severity, minimally expressed hemodynamic disturbances and diffuse inflammatory polymorphocellular infiltration were determined in the mucosa. In cases when patients suffered a severe COVID-19, we determined focal alterative-desquamative changes in the epithelium, single acute erosions, moderate hemodynamic disturbances, and diffuse inflammatory polymorphocellular infiltration in the mucosa. A comparative analysis showed, as in the previous post-COVID-19 period, increasing severity of general pathological processes in the mucosa with intensifying severity of COVID-19 in anamnesis.

Histological analysis of the mucous membrane of small and large intestine in patients with COVID-19 and in

post-COVID-19 period revealed, firstly, more expressed general pathological processes in patients with COVID-19 compared to those in post-COVID-19 period, and secondly, a decreasing severity of general pathological processes with longer duration of the post-COVID-19 period. Only patients with mild COVID-19 in anamnesis had completely recovered mucous membrane of small and large intestine when the post-COVID-19 period lasted from 51 to 100 days, as indicated above.

From the authors' point of view, changes in the morphology of the mucous membrane of small and large intestine in patients with COVID-19 and in post-COVID-19 period are caused by the receptor-mediated penetration of SARS-CoV-2 into the intestinal mucosa, followed by the induction of alterative changes in epithelial layer by this pathogen, inflammatory process, hemodynamic and trophic disorders.

ACE2 is known as an important regulator of small and large intestine homeostasis. Interestingly, this enzyme is more expressed in the gastrointestinal tract than in the respiratory system [16]. Damage of ACE2 leads to disruption of the morphofunctional state of the intestine, which is, among other things, the cause of inflammatory changes in it [17].

In our study in the comparison group, in patients with COVID-19 and in post-COVID-19 period, ACE2 expression was detected mainly on the apical surface of the surface epithelium of small and large intestine mucosa. This was more expressed in the small intestine compared to the large intestine, which is consistent with the literature data [18].

SARS-CoV-2 penetrates into the human body cells that have ACE2 receptors on the surface [19]. Our study and literature data [20] showed damage of this enzyme in COVID-19, characterized by a decrease in its amount and its activity. In this study, the authors found that the decrease in ACE2 content in the mucous membrane of small and large intestine increased with growing severity of COVID-19.

In cases of mild COVID-19 in anamnesis, when the duration of post-COVID-19 period lasted from 1 to 50 days, there was an increase in the content of ACE2 which, from our point of view, was of a compensatory nature. In persons who recovered from moderate and, especially, severe COVID-19, the authors found a deficiency of this enzyme.

In cases where the duration of post-COVID-19 period lasted from 51 to 100 days, the content of this enzyme corresponded to the norm in people who had mild COVID-19. However, in cases of moderate and, especially, severe COVID-19, we found a decreasing content of this enzyme.

The comparative analysis conducted by the authors showed that ACE2 deficiency was more pronounced in patients with COVID-19 of moderate and severe severity compared with patients in post-COVID-19 period of various durations who had this infection of similar severity. In patients who underwent moderate and severe COVID-19, ACE2 deficiency decreased with an increase in the duration of post-COVID-19 period. In patients who recovered from mild COVID-19, the ACE2 content was elevated in cases of the duration of post-COVID-19 period from 1 to 50 days, and it corresponded to the norm in cases of the duration of this period from 51 to 100 days.

Thus, the comprehensive morphological study conducted by the authors made it possible, firstly, to clarify the morphological and functional features of the mucous membrane of small and large intestine in patients with COVID-19 of various degrees of severity; secondly, to obtain new data about the morpho-functional state of the mucous membrane of small and large intestine in patients, taking into account different duration of the post-COVID-19 period and the severity of the infection.

CONCLUSIONS

1. The mucous membrane of small and large intestine in patients with COVID-19 is characterized by a decrease in the content of ACE2, alterative-desquamative changes in the epithelial layer, formation of acute erosions and ulcers, hemodynamic disorders and presence of diffuse inflammatory polymorphocellular infiltration. The changes noted by the authors in the mucosa of small and large intestine increase with growing severity of COVID-19.
2. In cases of post-COVID-19 period duration from 1 to 50 days in patients who recovered from mild infection, an increase in the content of ACE2, minimally expressed hemodynamic disturbances and diffuse polymorphocellular inflammatory infiltration are found in the mucous membrane of small and large intestine. In cases of a moderate severity infection in the mucosa the authors determine a decreasing content of ACE2, moderately expressed diffuse alterative-desquamative changes in the epithelial layer, signs of circulatory disorders and diffuse polymorphic cell infiltration. In cases of severe COVID-19 in the mucous membrane there is ACE2 deficiency, extremely expressed alterative-desquamative changes in the epithelium, hemodynamic disturbances and diffuse polymorphocellular infiltration, erosive and ulcerative processes.
3. In the duration of post-COVID-19 period from 51 to 100 days in people who have had a mild infection, the mucous membrane of small and large intestine is completely restored and corresponds to the norm. In cases where patients have had COVID-19 of moderate severity, a decreasing content of ACE2, minimally expressed hemodynamic disturbances, and diffuse inflammatory polymorphocellular infiltration are determined in the mucosa. In cases of severe infection, the mucosa is characterized by ACE2 deficiency, presence of focal alterative-desquamative changes in the epithelium, single acute erosions, moderate hemodynamic disturbances, and diffuse inflammatory polymorphocellular infiltration.
4. Analyzing the survey microscopy of the mucous membrane of small and large intestine in patients with COVID-19 and in post-COVID-19 period, the authors

were able to identify, firstly, more expressed general pathological processes in patients with COVID-19 compared with persons in post-COVID-19 period; and, secondly, a decreasing severity of general pathological processes with the increasing duration of the post-COVID-19 period.

5. The comparative analysis showed a more expressed deficiency of ACE2 in the mucous membrane of small and large intestine in patients with moderate and severe COVID-19 compared with patients in post-COVID-19 period of different duration. In patients who had moderate and severe COVID-19 in anamnesis, ACE2 deficiency decreases with increasing duration of post-COVID-19 period. In patients recovered from mild COVID-19, the ACE2 content increases with the duration of post-COVID-19 period from 1 to 50 days and corresponds to the norm with the duration of this period from 51 to 100 days.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSESSMENT OF TYPES OF MYOCARDIAL DIASTOLIC DYSFUNCTION DEPENDING ON THE DEGREE OF CORONARY ARTERY LESION IN PATIENTS WITH NSTEMI

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ABSTRACT

The aim: To evaluate the types of myocardial diastolic dysfunction depending on the degree of coronary artery lesion in patients with NSTEMI.

Materials and methods: We examined 200 patients with NSTEMI aged 38 to 80 years, who were urgently hospitalized in the Vinnytsya Regional Clinical Center of Cardiovascular Pathology. All patients underwent echocardiography examination from 3 to 5 day of hospitalization after coronary angiography.

Results: We established that there were no significant gender and age differences in the types of diastolic dysfunction. However, we found that with an increase in the degree of damage to the coronary arteries and an increase in the number of affected arteries, the degree of severity of myocardial diastolic dysfunction increases and the probability of occurrence of more unfavorable types of dysfunction increases.

Conclusions: Determining the types of myocardial diastolic dysfunction in the early period of NSTEMI makes it possible to predict unfavorable variants of the course of the disease and prevent possible complications.

KEY WORDS: NSTEMI, coronary arteries, myocardial diastolic dysfunction

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INTRODUCTION

In the structure of general and cardiovascular morbidity and mortality worldwide, one of the leading causes is myocardial infarction [1], in particular, such forms as non-ST segment elevation myocardial infarction (NSTEMI). Improving diagnosis and finding more effective methods of treating myocardial infarction is a priority task of modern cardiology. Despite significant progress in the treatment of acute myocardial infarction in most developed countries, questions regarding the occurrence and prevention of early and late complications of infarction remain unanswered [2]. Due to the development of myocardial dysfunction on the background of post-infarction remodeling and the occurrence of prognostically unfavorable arrhythmias on the background of electrically unstable myocardium, the long-term prognosis of this category of patients remains disappointing [3]. The study of remodelling processes, their influence on the development of electrical instability of the myocardium and the search for possibilities of predicting the development of complications is currently considered a promising direction of non-invasive diagnosis of myocardial infarction [4]. An unfavorable long-term prognosis in the case of NSTEMI is primarily caused by the development of myocardial dysfunction, electrical instability of the myocardium, or the development of repeated ischemic coronary events [5].

THE AIM

The aim of the study is to evaluate the types of myocardial diastolic dysfunction depending on the degree of coronary artery lesion in patients with NSTEMI.

MATERIALS AND METHODS

All studies conform to the principles of the Declaration of Helsinki of the World Medical Association. The study protocol, the form of informed consent of patients and other documents related to the study were approved at the meeting of the Academic Council of the National Pirogov Memorial Medical University, Vinnytsya (excerpt from the protocol No. 2 from 27.02.2020). Informed consent to participate in the study was discussed and signed by all study participants. We examined 200 patients with NSTEMI aged 38 to 80 (mean 62.0 ± 0.71, median – 62 and interquartile range – 55 and 70) years, who were urgently hospitalized in the Vinnytsya Regional Clinical Center of Cardiovascular Pathology. The main criteria for inclusion of patients in the study were: NSTEMI, which emerged for the first time; age of patients up to 80 years and the patient's informed consent to participate in the study. The diagnosis of NSTEMI was established according to the recommendations of ESC, 2020 [6]. The criteria for exclusion from the study were: 1) STEMI, transferred in the past and recurrent acute myocardial infarction; 2) age of patients 80 years and

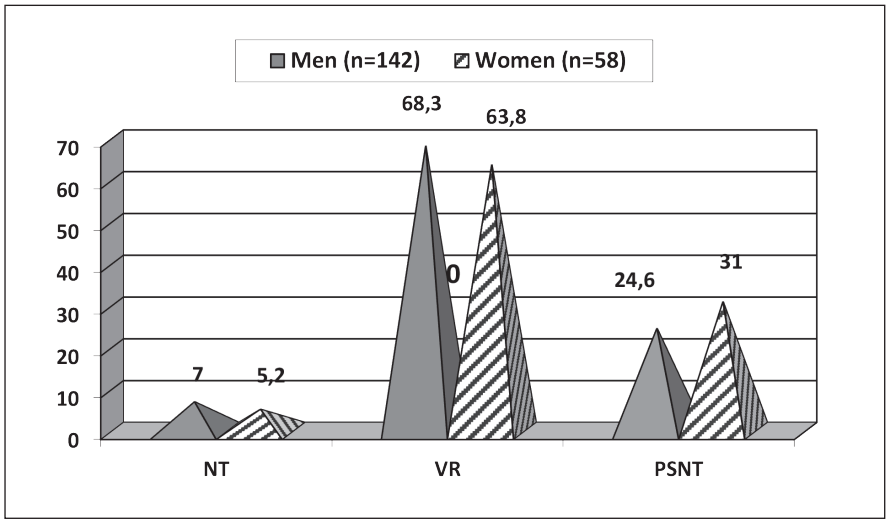

Fig. 1. Character of diastolic transmitral blood flow in NSTEMI patients depending on gender
Notes: 1. The distribution of types of diastolic transmitral blood flow is given in %: NT – normal type, VR – violation of relaxation; PSNT is a pseudo-normal type;
2. The statistical reliability of the difference in percentages between groups was calculated according to the χ2 criterion

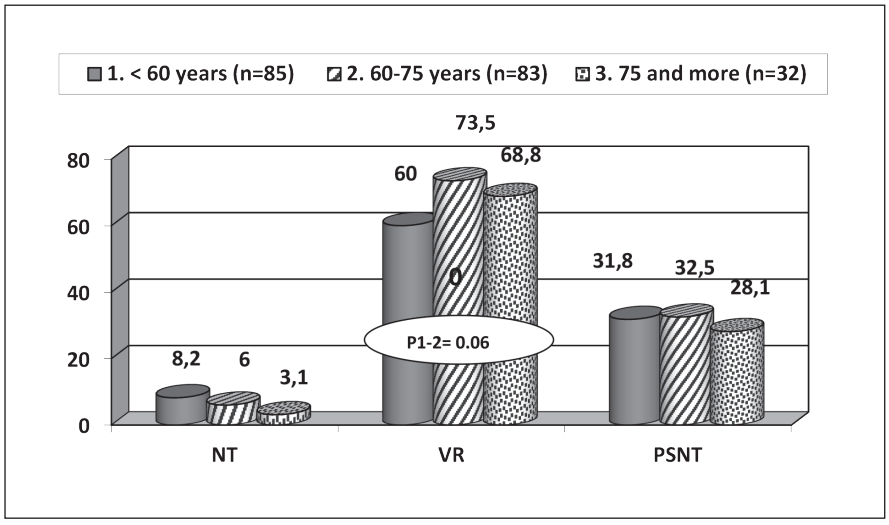

Fig. 2. Character of diastolic transmitral blood flow of NSTEMI patients depending on age
Notes: 1. The distribution of types of diastolic transmitral blood flow is given in %: NT – normal type, PR – violation of relaxation; PSNT – pseudonormal type;
2. The statistical reliability of the difference in percentages between groups was calculated according to the χ2 criterion

older; 3) the presence of sinoatrial or atrioventricular block II-III degree, implanted or the need for implantation of an artificial pacemaker; 4) chronic heart failure NYHA-III, IV before the incident of acute myocardial infarction; 5) diseases of the respiratory system, kidneys and liver, which were accompanied by signs of pulmonary, renal and hepatic failure; anemic conditions with a hemoglobin level below 110 g / L; 6) the presence of rheumatic and congenital heart defects, idiopathic and inflammatory myocardial lesions and 7) malignancies, severe neuropsychiatric disorders, alcohol abuse. All patients underwent echocardiography examination in M-, B-, D-modes from 3 to 5 day of hospitalization after coronary angiography.

RESULTS

The analysis of different types of diastolic transmitral blood flow in NSTEMI patients showed (Fig. 1) that, as a whole, 134 (67.0%) of the examined subjects had left ventricle (LV) relaxation disorders (type I diastolic dysfunction), 53 (26.5%) had pseudonormal (II type) and only in 13 (6.5%) - absence of pathological changes (normal type) of diastolic transmitral blood flow.

The analysis performed depending on the gender of the patients demonstrated the absence of any significant ($p>0.40$) intergroup differences (Fig. 1). Therefore, it was necessary to state the fact that in the majority of subjects examined with NSTEMI, according to the echocardiogram, diastolic transmitral blood flow disorders were determined according to the variant of relaxation disorders. At the same time, we found no significant differences in the nature of diastolic transmitral blood flow in different gender groups of patients.

It was found that in people aged 65-70, compared to patients under 60, a significant increase in cases with a Ve/Va ratio < 1.0 (60.2% vs. 36.5%, $p=0.002$) and a decrease in cases with Ve /Va -1.0-1.5 (24.1% vs. 40.0%, $p=0.03$, respectively). Therefore, the obtained data indicated a certain association of the age of NSTEMI patients with the severity of diastolic disorders of the LV myocardium, which was characterized by corresponding changes in the Ve/Va ratio.

Analysis of diastolic transmitral blood flow in NSTEMI patients depending on the selected age group (Fig. 2) demonstrated the absence of significant differences between groups of patients. We determined only a trend with a tendency to reliability between the groups of patients

Table I. Myocardial diastolic function in NSTEMI patients depending on the presence of hemodynamically significant CA stenoses

Echocardiogram indicators (n=156)	There is no HSS CA (n=22)	Available HSS CA (n=134)	P
Ve/Va	0,94 (0,79; 1,08)	0,96 (0,83; 1,44)	<0,05
Ve/Va < 1,0, n (%)	12 (54,5%)	71 (53,0%;)	<0,05
Ve/Va - 1,0-1,5, n (%)	10 (45,5%)	36 (26,9%)	<0,05
Ve/Va > 1,5, n (%)	0 (0)	27 (20,1%)	0,02

Note. Comparison of percentages between groups was carried out according to the χ2 criterion, absolute values - according to the Mann-Whitney U test

Table II. The structural and functional state of the myocardium in NSTEMI patients depending on the severity of the anatomical lesion of the CA

Echocardiogram indicators (n=156)	Total score of CA damage ≤ 3 (n=97)	Total score of CA damage > 3 (n=59)	P
Ve/Va	0,91 (0,80; 1,07)	1,09 (0,88; 1,58)	0,04
Ve/Va < 1,0, n (%)	59 (60,8%)	24 (40,7%)	0,01
Ve/Va - 1,0-1,5, n (%)	30 (30,9%)	16 (27,1%)	0,61
Ve/Va > 1,5, n (%)	8 (8,2%)	19 (32,2%)	0,0001

Note. Comparison of percentages between groups was carried out according to the χ2 criterion, absolute values - according to the Mann-Whitney U test

under 60 and 60-75 years of age in terms of the frequency of registration of the most common type – impaired LV relaxation (60.0% vs. 73.5%, p=0.06).

In order to assess the severity of damage to individual main arteries, we calculated a conditional score for coronary artery (CA) damage, where 0 points are the absence of any atherosclerotic plaques in the CA (intact artery), 1 point is the presence of atherosclerotic stenosis up to 50%, 2 points are from 50 % to 90% of the CA and 3 points – CA occlusion (atherosclerotic stenosis > 90%). In addition, the total conditional CA damage score was additionally calculated as the sum of the right coronary artery (RCA), left anterior descending artery (LAD) and left circumflex artery (LCx) damage scores. According to the obtained data, it was observed that the average conditional score of RCA damage was 0.74±0.08, LAD – 2.03±0.10 and LCx – 0.89±0.09, respectively. At the same time, the total conditional score of CA damage was 3.66±0.17 [7].

The analysis of the results of the Echocardiogram study of NSTEMI patients, depending on the nature of the anatomical lesion of the CA, is shown in the table. I. To conduct the analysis, we used two methodologies for the selection of groups: 1st – groups with absent (n=22) and present hemodynamically significant stenosis/s (HSS) of the CA (n=134) were selected and 2nd – groups with conditionally mild/moderate (n=97) and severe anatomic lesions of the CA (n=59) according to the total score of the severity of the CA damage.

The analysis of diastolic transmitral blood flow (Fig. 3) showed that in patients with NSTEMI without HF, in contrast to the group with HF, a significant increase in normal blood flow (22.7% vs. 0, p=0.002) and a decrease in pseudonormal type (0 against 25.4%, p=0.008).

In the table II presents the following analysis of Echocardiogram indicators in NSTEMI patients depending on the severity of CA damage.

To select the comparison groups, the total score of the severity of the anatomical lesion of the CA, proposed by us, was taken, the method of calculation of which is given above. Therefore, we selected two comparison groups: 1st - patients with a total score ≤ 3 (conditionally mild/moderate, n=97) and 2nd - with a total score > 3 (severe CA damage, n=59), where 3 is the median of the total score of CA damage severity, which was calculated as a whole for the group of NSTEMI patients according to coronary angiography data (n=156).

The obtained data showed that in the group with more severe (total score > 3), compared to the group with mild/moderate CA damage (total score ≤ 3), the Ve/Va ratio was observed (1.09 vs. 0.91, p=0 .04), Ve/Va ratio > 1.5 (32.2% vs. 8.2%, p=0.0001) frequency of cases with Ve/Va ratio < 1.0 (40.7% vs. 60.8% , p=0.01).

The analysis of the nature of diastolic transmitral blood flow in NSTEMI patients depending on the severity of the anatomic lesion of the CA (Fig. 4) showed a clear association between the nature of the CA lesion and impaired diastolic function of the LV myocardium. Thus, it was found that in the group with a total CA lesion score > 3, compared to the group of patients with milder CA lesions, a significant decrease in the normal type (0 vs. 11.3%, p=0.007) and an increase in the pseudonormal type (40.7 % versus 10.3%, p<0.0001), as the most problematic type of LV diastolic dysfunction, identified by us in the examined cohort of patients.

On the other hand, a significant decrease in cases of impaired relaxation (59.3% vs. 78.4%, p=0.01), as the most

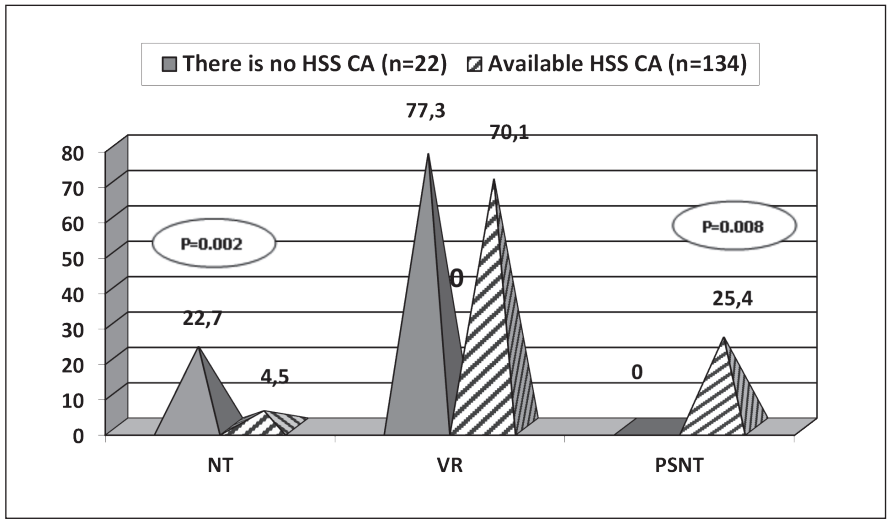

Fig. 3. Character of diastolic transmitral blood flow in NSTEMI patients depending on the presence of hemodynamically significant CA stenoses
Notes: 1. HSS CA - hemodynamically significant stenosis of the coronary artery;
2. The distribution of types of diastolic transmitral blood flow is given in %: NT – normal type, VR – violation of relaxation; PSNT – pseudonormal type;
3. The statistical reliability of the difference in percentages between groups was calculated according to the χ2 criterion

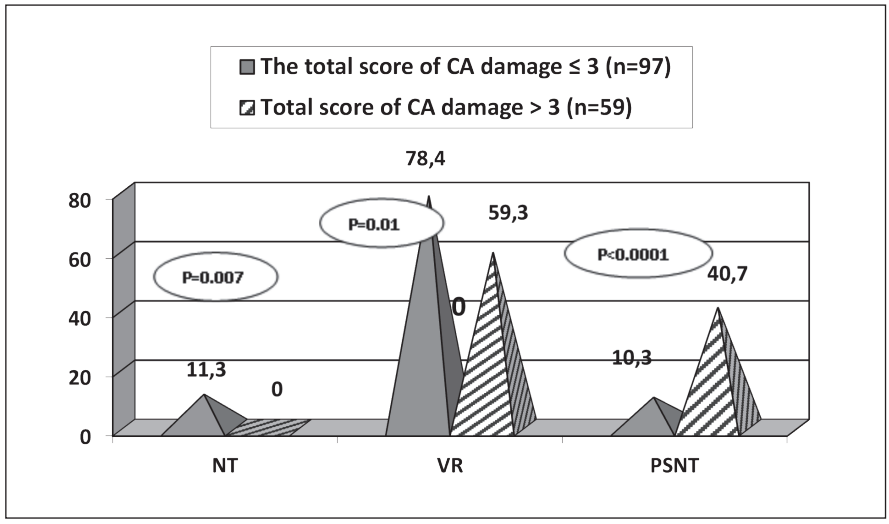

Fig. 4. Character of diastolic transmitral blood flow in NSTEMI patients depending on the severity of the anatomical lesion of the coronary artery
Notes: 1. The distribution of types of diastolic transmitral blood flow is given in %: NT – normal type, VR – violation of relaxation; PSNT is a pseudo-normal type;
2. The statistical reliability of the difference in percentages between groups was calculated according to the χ2 criterion

frequent type of LV diastolic dysfunction, in patients with more severe CA damage should be explained by a significant increase in cases with pseudonormal type, which was mentioned above.

DISCUSSION

We established a relationship between the degree of CA stenosis and manifestations of diastolic dysfunction of the left ventricle. A number of previous studies compared the manifestations of diastolic dysfunction both in patients with NSTEMI and in other manifestations of coronary heart disease, in particular, in unstable angina [8]. Also, the absence of gender and age specific manifestations of diastolic dysfunction in the selected category of patients was established, while other studies demonstrate the presence of similar relationships [9]. In our opinion, this is related to different statistical samples taken into account during the research. Only the fact of a positive correlation between the degree of coronary artery damage and manifestations of diastolic myocardial dysfunction is indisputable. Therefore, the determination of the types of myocardial diastolic dysfunction in the early period of NSTEMI allows predicting adverse variants of the course of the disease and preventing possible complications.

CONCLUSIONS

1. There are no significant gender-age differences in the manifestations of diastolic dysfunction in patients with NSTEMI.
2. With an increase in the degree of damage to the coronary arteries and an increase in the number of their damage, the progression of diastolic myocardial dysfunction to its most prognostically unfavorable manifestations is noted.

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ORIGINAL ARTICLE

FEATURES OF THE ACETABULUM POSTERIOR WALL FRACTURES DELAYED SURGICAL TREATMENT

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ABSTRACT

The aim: To analyse the results of the delayed acetabulum posterior wall fractures treatment and to identify the negative factors affecting the outcome.

Materials and methods: The patients' treatment results have been assessed in 5 years after the surgery. The surgery was performed in 21 to 120 days after the injury, 44±11 days on average. 23 individuals (19 men and 4 women) were included in the retrospective analysis.

Results: Anatomical fracture reposition was achieved in 12 (52.2%) cases, imperfect but satisfactory reposition – in 7 (30.4%) cases. 4 (17.4%) cases resulted in unsatisfactory reposition. Hip arthroplasty was performed in 5 patients over the following 5 years.

Conclusions: The delayed fractures of the acetabulum posterior wall (21 to 120 days after the injury) is the complicated task for a surgeon. The aseptic necrosis and deforming arthrosis were found in 1-5 years after treatment. The preparation for the anatomical reposition takes a big amount of time and effort from the orthopedic team because of technical peculiarities.

KEY WORDS: acetabulum posterior wall, fracture, delayed treatment

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INTRODUCTION

Acetabulum fractures are relatively rare injuries (3/100 000 per year) [1] and are mostly distributed bimodally: high-energy injuries and low-energy injuries [2]. Dislocated acetabulum fractures are high-energy injuries that are associated with polytrauma, including head trauma, chest trauma, spine, abdomen, and pelvis injuries, the opened fractures of upper and lower extremities. Such concomitant life-threatening conditions and open wounds treatment has unequivocal priority over radical surgical treatment in case of acetabulum fractures. The fracture fixation in these cases may be delayed. Medical optimizing the patients who suffered multiple injuries may sometimes takes longer than expected due to physiological response to combined multiorgan injuries, which delays the acetabulum fractures osteosynthesis. The patients' delayed transfer from secondary hospitals may sometimes lead to the significant delays in the surgical treatment of these patients [3,4].

Surgical reconstruction of fresh displaced acetabulum fractures has become a conventional method in order to achieve the best long-term results [5-13]. The acetabulum posterior wall fractures should be surgically treated relatively early when the patient's condition has been stabilized and the surgical complications risk has been minimized [11-13]. Surgical treatment of the acetabulum posterior wall fractures can result in good and excellent results in more than 80% of cases, given that patients' condition is stabilized within 14 days after injury, the femoral head dislocation is eliminated and the fracture fragments are anatomically repositioned [3, 10-13]. If the amount of time from the injury to the reposition and stabilization exceeds 21 days, some changes occur in the surrounding soft tissues, the muscles contract, the amount of scar tissue between bone fragments increases. Letournel and Judet [5-9,11] has divided the acetabulum fracture surgeries into 3 post-injury periods: up to 21 days, 21–120 days, and more than 120 days after the injury. The fractures stabilized in the first period are classified as a delayed treatment and are usually characterized by some easy to recognize fracture lines that help achieve reposition and perform osteotomy if needed. After 120 days the fracture lines become blurred which leads to incorrect consolidation becoming the most significant problem during the surgical correction.

THE AIM

The aim of this study was to analyse the results of the delayed acetabulum posterior wall fractures treatment and to identify the negative factors affecting the treatment outcome.

MATERIALS AND METHODS

In this study we relied on the patients' treatment results evaluation 5 years after surgery. The surgery was performed 21 to 120 days after the injury, 44±11 days on average. The retrospective analysis included 23 individuals (19 (82.6%) men and 4 (17.4%) women) with old acetabulum fractures who underwent surgical treatment in 2011-2017. The patients' age varied from 26 to 62 years, 38 ± 5 years on average. 18 (78%) people were injured in car accidents, and 5 (22%) suffered from catatrauma. Patients' distribution in accordance with the Letournel-Judet classification: posterior wall fracture was diagnosed in 15 (65.2%) patients, posterior column fracture – in 3 (13.0%), posterior column and posterior wall – in 5 (21.8%). Patients' distribution according to the complications' presence: hip dislocation – in 20 (87%) cases, femoral head contusion – in 5 (21.7%) cases, acetabulum posterior wall comminuted fracture – in 13 (56.5%) cases. Femoral dislocation was fixed on the first day in 15 (65%) patients, on 2nd-3rd day - in 3 (15%) patients, later – in 2 (10%) patients. Recurrent hip subluxation during the surgery was observed in 7 (30.4%) patients.

The Kocher-Langenbeck approach was used in 17 (73.9%) patients, the extended iliac-femoral approach was used in 6 (26.1%) patients.

The post-operative evaluation of the reposition was performed using the Matta scale: anatomical (displacement up to 1mm), imperfect (2-3mm), unsatisfactory (more than 3mm). The treatment results were assessed according to the Harris scale and the Merle d'Aubigné and Postel evaluation system [14].

RESULTS

The anatomical reposition was achieved in 12 (52.2%) cases, imperfect reposition – in 7 (30.4%) cases, unsatisfactory reposition – in 4 (17.4%) cases. Early complications included: post-traumatic neuropathy – 1 (7%) patient, intraoperative bleeding – 1 (7%) case, due to superior sciatic artery damage. Late complications included the femoral head aseptic necrosis that was observed in 21 (91.3%) patients over a 5-year period.

The delayed fixation of the hip dislocation or the femoral head recurrent subluxation resulted in observing the aseptic necrosis in 8 (89%) of 9 patients over a 5-year period. The femoral head aseptic necrosis was observed in 5 patients (100%) out of 5 with its lesion. The hip joint deforming osteoarthritis was present in 18 (78%) people during the observation stages. Aseptic necrosis or deforming arthrosis of the hip joint was detected in all 4 patients with unsatisfactory repositioning. Hip arthroplasty was performed in 19 (82.6%) patients over the 5-year period.

Treatment results calculation according to Harris scale: initially – 87±11 points, in 1 year – 77±12 points, after 5 years – 85±10 points. This indicates a decrease in the patient's life quality over time. These results were influenced by the neuropathy, aseptic necrosis, and deforming arthrosis development. The results' improvement after 5 years happened due to the hip arthroplasty in 19 (82.6%) patients.

The pain syndrome and motion range were assessed in the operated patients as well as their ability to move using the Merle d'Aubigné and Postel calculation system (0-6 points scale, where 6 is the best indicator for each criterion: hip joint pain and mobility, walking ability assessment; the best total score – 18 points, the worst – 0 points). For mobility evaluation passive movements in the hip joint were performed and measured with a universal goniometer (Table I).

Evaluation of treatment results according to the Merle d'Aubigné and Postel scale: initially – 16.7 points, in a year – 14.9 points, in 5 years – 16.5 points.

DISCUSSION

Surgical treatment of the old fractures is not as easy as one can think. Unfortunately, having fixed the dislocation, orthopedists treat a lot of patients with skeletal traction for a big amount of time, thus underestimating the fragments and femoral head reposition after fixing the dislocation and sending the patient to a specialized department only in 3-4 weeks. The reason for this is often X-Ray or CT control

Table I. Merle d'Aubigné and Postel scale

Score	Pain	Mobility	Walking ability
0	Intense and permanent	Ankylosis in abnormal position	Impossible
1	Severe, disturbing sleep	Ankylosis in normal position or in slightly abnormal position	Only with crutches
2	Severe when walking, limits activity	Flexion < 40° (abduction = 0°) or very light joint deformity	Only with two canes
3	Severe but allows limited activity	Flexion < 40°– 60°	Limited with one cane (less than one hour). Very difficult with no canes
4	Moderate when walking, disappearing with rest	Flexion > 60°– 80° (can tie shoelaces)	Prolonged with one cane, limited with no canes (limp)
5	Very little pain and intermittent, doesn't preclude normal activity	Flexion > 80°– 90°, limited abduction (>25°)	With no canes but slight limp
6	No pain at all	Normal. Flexion > 90°, abduction >25°	Normal

scans that should have been done immediately after fixing the dislocation. The errors also often occur as far as in the X-Ray or CT interpretation. Orthopedists forget that the evaluation cannot be made only with the anterior-posterior X-Ray and omit the additional X-ray projections by Judet.

Muscle and capsule retraction, ossification, and scarring in the fracture area are major difficulties in the surgical treatment of such injuries. Therefore, even simple fracture models may require more advanced approach with less chances of anatomical reposition.

As well as in case of acute acetabulum fractures, preoperative planning is crucial for understanding the type of fracture, determining surgical approach, assessing the reconstruction complexity, creating a surgical plan, and for a long-term prognosis in the end. Standard radiographic evaluation includes anterior-posterior projection and oblique projection by Judet, as well as computer tomography (CT). The most important additional radiological technique is the creation of 3D images for improving the understanding of fracture type, amount of bone callus, ossifications, and important neurovascular structures positions. In most cases of isolated acetabulum posterior wall or column fractures, the Kocher-Langenbeck approach was used, as it provides an adequate exposure even after long delays in treatment. The problem with the approach is the inability to adequately release the anterior capsule of the joint in case the femoral head dislocation wasn't fixed.

In addition to the peculiarities of the approach, we would like to remind you of using the orthopedic table features in order to achieve proper traction during repositioning and fixation, as well as the tools that can allow you to successfully reposition the posterior wall fragments without destroying it. Scar tissue between the fracture fragments should be removed to achieve reposition. Exposing the fracture fragment from scar tissue should be avoided for maintaining vascularization. The impacted fragment areas require a special attitude. The fragments can often be hard to isolate, and the zone is often hard to restore. Using supporting bone plastic from the acetabulum or iliac bone ala is important while performing this [5].

Different types of fixations will also be required in the osteosynthesis of old fractures, comparing to the osteosynthesis of fresh fractures. Using screws or short reconstructive plates will no longer be suitable. Massive long pelvic plates and "springs" instead of small cortical screws in case of fresh comminuted posterior wall fractures. Two plates instead of one may be required for better stability.

CONCLUSIONS

1. Factors that may have an influence on the delayed treatment of acetabulum posterior wall include femoral head damage (radiological and intraoperative assessment) – in 100% of cases; late elimination of the femoral head dislocation – in 10% of cases; recurrent subluxation – in 30.4% of cases; anatomical reposition – in 52.2% of cases.
2. The results of acetabulum posterior wall fractures delayed treatment are significantly different to the ones treated with early osteosynthesis. The reasons for that are difficulties in anatomical repositioning, femoral head malnutrition due to its displacement or chronic subluxation, reduced blood supply to the fragments due to surgical manipulations during the approach.
3. The deterioration of the delayed treatment results was observed withing 1 to 5 years after the surgery due to the aseptic necrosis and deforming arthrosis development. The Harris scale and the Merle d'Aubigné and Postel evaluation systems showed the improvements in treatment results after the hip replacement surgeries were performed in the delayed period.
4. Delayed treatment of the acetabulum posterior wall fractures (21 to 120 days after injury) is a complicated task for a surgeon. The anatomical reposition in these cases is a problem solving which requires a lot of time and technical effort from the orthopedists team and should be performed in specialized departments that have work experience with different types of fractures.

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ORIGINAL ARTICLE

CLINICAL PECULIARITIES OF WOUND HEALING AFTER SURGICAL TREATMENT OF PATIENTS WITH GENERALIZED PERIODONTITIS ACCOMPANIED BY DIFFERENT TYPES OF REACTIVITY OF THE BODY

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ABSTRACT

The aim: To study the features of clinical wound healing (intensity and duration of inflammatory reaction) after the indications for patients with generalized periodontitis II, III degrees of severity of patch surgery accompanied by normo-, hyper- and hyporeactivity of the body.

Materials and methods: 216 people aged 45 between 55 years with a diagnosis of generalized periodontitis of II, III degree of severity, chronic course were examined. Depending on the condition of reactivity of the organism, the patients were divided into three groups: the first one consisted of people with normoreaction; the second group included patients with hyperreaction; the third group was made up by people with hyporeaction. The patients after the initial therapy, underwent patch surgery. Initially, on the 2nd, 4th, 6th and 9th day after the operation, the clinical examination of the periodontal tissues (examination, hygienic and periodontal indices) was performed. Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0.

Results: Normoreactivity of the body determines the normal time of wound healing after patch surgery, which on the 9th day is characterized by the clinical condition of the gingival mucosa, which corresponds to the concept "healthy" for 98% of patients. In case of hyperreactivity of the organism slowing down of processes of wound healing for 3-4 days in comparison with the time which is typical for normoreactivity with dynamics of the indices which specifies long intensive character of inflammatory reaction in the postoperative period has been observed. In case of hyporeactivity of the body as well as in case of hyperreaction, there was a slowdown in wound healing for 3-4 days compared with normoreactivity with the dynamics of indices that showed slow sluggish nature of the inflammatory reaction in the postoperative period.

Conclusions: Correction of altered parameters in patients with generalized periodontitis accompanied by impaired (hyper- and hypo-) reactivity of the body with bringing them to values which are typical for normoreactivity is considered to be a condition for optimizing mucosal wound healing after surgery and further stabilization of the periodontal tissues.

KEY WORDS: periodontitis, reactivity of the organism, clinical indicators, wound healing

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INTRODUCTION

One of the leading places in the structure of dental diseases is occupied by generalized periodontitis, which is connected with its widespread [1]. The importance of the problem of inflammatory and destructive periodontal disease is preconditioned by its socio-economic significance, namely: a significant increase in destructive forms of people of young, working age, with high recurrence rate, the consequences leading to tooth loss and dysfunction of the dental system, the negative impact on the body as a whole [2,3]. It is a well-known fact that generalized periodontitis is a pathological process that develops in periodontal tissues because of the combined influence of various general and local exogenous and endogenous factors [4,5]. At the same time a number of neuro-regulatory, neuro-trophic, biochemical, immunological and functional disorders, microcirculatory and metabolic disorders, disorders of almost all the types of metabolism develop: protein, lipid, carbohydrate, mineral, which eventually leads to irreversible destruction of the periodontium and the alveolar bone [6-8].

In connection with the above-mentioned facts, the search for new tools, methods and approaches to the complex treatment of generalized periodontitis remains very important. General and local drug therapy has been widely used in the complex treatment of this pathology [9-11]. The correctness of this tactic is confirmed by numerous data from the literature sources on the high clinical effectiveness of the suggested and implemented drug treatment regimens for generalized periodontitis [12-14]. However, the question of clarifying the key mechanisms of development and course of the disease remains relevant, in particular - depending on the state of reactivity of the organism, which has a significant impact on the pathogenesis of generalized periodontitis. There is no doubt that the clinical features of the inflammatory process (its intensity and duration) are preconditioned by the state of reactivity of the organism [15].

Table I. The initial condition of periodontal tissues in patients with generalized periodontitis of II, III degree of severity accompanied by normo-, hyper- and hyporeactivity of the organism before the surgical treatment

Indices	Groups of patients		
	normoreaction	hyperreaction	Hyporeaction
Colour of gums	stagnant hyperaemia	pronounced hyperaemia	Bluish
Swelling of gums	not pronounced	pronounced	Loose
Amount of exsudate, mm^2	4,28 ± 0,34	6,11 ± 1,35 «	3,42 ± 1,02»
Index PMA,%	76,7 ± 2,4	84,4 ± 5,1 «	70,4 ± 5,8»
PI Russel	4,9 ± 0,28	5,2 ± 1,51	5,1 ± 1,49
HI according to Fedorov / Volodkina, points	1,8 ± 0,24	2,9 ± 1,45»	2,8 ± 1,46»

Note: "- p <0,05 against the values which are typical for normoreactivity of the organism

Table II. The condition of periodontal tissues in patients with generalized periodontitis of II, III degree of severity accompanied by normo- (1), hyper- (2) and hyporeactivity (3) of the body after surgical treatment

Indices	Terms of Observation								
	The 2nd day			The 4th day			The 6th day		
	1	2	3	1	2	3	1	2	3
Pain, (abs.fr /%)	20/15	5/20	3/16	2/2	3/13	1/5	-	1/4	-
Hyperaemia, (abs.fr /%)	132/100;23/100;19/100			132/100;23/100;19/100			53/40 12/52 9/47		
Oedema, (abs.fr. /%)	132/100;23/100;19/100			132/100;23/100;19/100			61/4 15/65 11/58		
Amount of exsudate, mm2	5,32 0,60	7,42 1,82*	4,45 1,52*	4,20 0,47»	6,82 1,71	4,35 1,49	2,43 0,26»	3,90 1,28»*	3,46 1,25»*
PMA, %	82,4 2,9	89,1 5,3*	75,8 5,6*	51,6 2,1 «	69,4 4,5»*	65,3 4,6 «*	22,0 0,9»	39,3 2,8 «*	35,2 2,9»*
Pisarev's probe	+++	+++	++	++	+++	++	-	+	+

Note: « - p <0.05 against the values on the 2nd day

* - p <0,05 against values which are typical for normoreactivity of the organism

THE AIM

The aim of this research was to study the features of clinical wound healing (intensity and duration of the inflammatory reaction) of patients with generalized periodontitis of II, III degrees of severity accompanied by normo-, hyper- and hyporeactivity of the body after the patch surgery according to the indications.

MATERIALS AND METHODS

216 patients (82 men and 134 women), aged 45 between 55 years, with the diagnosis of generalized periodontitis of II, III degree of severity, chronic course were examined. The diagnosis was made on the basis of clinical examination, radiography, determination of periodontal samples in accordance with the International Classification of Diseases ICD-10. Depending on the state of reactivity of the body the patients were divided into three groups: the first one included patients with normoreaction (132 people, 61%); the second group consisted of patients with hyperreaction (46 people, 21%); the third one contained patients with hyporeaction (38 people, 18%). The division of patients into groups depending on the state of reactivity of the organism was performed on the basis of the identified clinical and laboratory differences. All the patients underwent comprehensive treatment of generalized periodontitis in the amount recommended by the Ministry of Health of Ukraine - Order №566 from 23.11.04. "On approval of the Protocols for the Provision of Medical Care". All the patients with generalized periodontitis of II, III degrees of severity after completion of the complex of initial therapy, according to the indications, underwent patch surgery. Initially, on the 2nd, 4th, 6th and 9th day after surgery, the clinical examination of periodontal tissues was performed. Much attention was paid to the color, tightness, relief of the marginal edge of the gums, the presence of edema, the severity of redness, pain. In order to assess the condition of the periodontium objectively, the determination of hygienic, periodontal indices and samples was performed:

- hygienic index according to Fedorov-Volodkina (on the five-point scale);
- papillary-marginal-alveolar index of PMA (in% - from 0 to 100%);
- intensity of exudation from periodontal pockets (in mm2);
- periodontal index (PI) Russell (in points - from 0 to 8);

- Schiller-Pisarev's test (visual qualitative assessment of the intensity of staining of the oral mucosa).

Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0 (STA862D175437Q).

RESULTS

Initially, the condition of periodontal tissues in patients with generalized periodontitis with normoreactivity of the organism corresponded to II and III degree of severity, chronic course (Table I).

This is evidenced by stagnant hyperemic, slightly swollen mucous membrane of the oral cavity and the corresponding values of paraclinical indices - the amount of exudate from periodontal pockets, PMA and PI Russell. According to Fedorov-Volodkina, the hygienic condition of the oral cavity was characterized as "unsatisfactory".

After the surgery (according to the indications of patch surgery) on the 2nd day in patients with generalized periodontitis accompanied by normoreaction, the values of indicators reflecting the intensity and prevalence of the inflammatory process were characterized by the corresponding dynamics (Table II).

Thus, the appearance of pain in the wound was noticed by 15% of patients. Hyperemia and edema of the gingival mucosa were registered in all the patients which were examined. More objective confirmation of the intensification of the inflammatory response in response to "damage" is the result of the assessment of paraclinical indices. Namely: significant exudation from periodontal pockets was noticed (the amount of inflammatory exudate reached 5.32 ± 0.60 mm2); high values of the PMA index characterized the significant prevalence of gingivitis; the intensity of the inflammatory reaction according to Schiller-Pisarev's probe corresponded to the "pronounced" (the mucous membrane of the oral cavity was painted in dark brown). Beginning with the 4th day after the intervention, despite the visually determined hyperemia and edema, the intensity and prevalence of the inflammatory reaction in the postoperative wound according to the index of assessment decreased significantly. Thus, the amount of exudate became 1.3 times lower ($p > 0.05$), the prevalence of the inflammatory process by the PMA index became 1.6 times lower ($p < 0.05$). On the 6th day in most patients at objective examination there were no signs of redness and swelling of the mucous membrane of the gums, respectively, in 40 and 46% of cases. The amount of exudate from periodontal pockets became 2.2 times lower compared with the level on the 2nd day after surgery ($p < 0,05$). Also there was the decrease in the prevalence of inflammation in the PMA index: it became 3.7 times lower ($p < 0,05$). On the 9th day, most of the patients had such a condition of the gingival mucosa that corresponded to the conception of "being healthy" (with the exception of 2 patients; 1.5%). The mucous membrane of the gums was pale pink, elastic, tightly covered the neck of the tooth, periodontal pockets are absent, the teeth were immobile.

The results of clinical examination of patients with generalized periodontitis with hyperreactivity showed that initially the condition of periodontal tissues, as in normoreactivity, corresponded to the diagnosis, which was confirmed by the clinical picture and the corresponding values of the studied indices (Table I).

It is necessary to take into consideration some differences compared with patients with GP with normoreaction of the body, namely: visually more pronounced redness and swelling of the gums, more pronounced intensity of the inflammatory process (the amount of exudate from periodontal pockets, $p < 0,05$) and its prevalence (according to the PMA index, $p < 0,05$). Hygienic condition of the oral cavity according to HI Fedorov-Volodkina in patients of the second group corresponded to "bad" ($p < 0,05$).

After the surgery, all the patients of the second group had redness and swelling of the gingival mucosa, as in case of normoreaction. (Table II).

The intensity of the postoperative inflammatory reaction was less pronounced compared with that typical for patients with GP of the first group, as evidenced by the "weakly positive" Schiller-Pisarev's test and the exudation from periodontal pockets which became 1,39 times higher ($p > 0.05$). The PMA index, which characterizes the prevalence of gingivitis, on the 2nd day was 1.1 times less pronounced in comparison with its corresponding value in patients with normoreactivity ($p > 0.05$). The reduction of signs of postoperative inflammation was slower compared to that typical for normoreactivity - began on the 4th day, but even on the 6th day the signs of inflammatory reaction persisted in half of the patients. There was a significant decrease in comparison with the 2nd day of exudation and PMA index, respectively, 1.9 and 2.2 times less ($p < 0,05$). However, despite the existing dynamics before the reduction of the inflammatory reaction, the amount of exudate and PMA index in patients of the third group remained significantly more pronounced in comparison with the corresponding value in the normal response of the body ($p < 0,05$). It is important that in contrast to patients of the first group, Schiller-Pisarev's test of the patients with reduced reactivity of the body remained "weakly positive" longer. All this indicates a slowdown in the healing of postoperative wounds in patients with GP accompanied by hyporeactivity of the body. On the 9th day, 12% of patients in the third group still noted the presence of an inflammatory reaction in the wound, which was clinically manifested by hyperemia and edema and the corresponding index values. Thus, in patients with generalized periodontitis accompanied by hyporeactivity of the body, clinical wound healing after surgery on periodontal tissues was delayed for 3-4 days in comparison with that of normoreactivity and similar to that of hyperreactivity of the body.

DISCUSSION

Our research confirms the key role of the body's reactivity in the healing of postoperative wounds in patients with generalized periodontitis who are undergoing surgical

intervention. Generalized periodontitis is an inflammatory-destructive process characterized by the development of a violation of almost all types of metabolism: protein, lipid, carbohydrate, mineral, which ultimately leads to the irreversible destruction of the periodontium and alveolar bone, leading to the loss of teeth [2]. Like any inflammatory process, in its course, generalized periodontitis has all phases of inflammation: alteration, exudation, and proliferation [6]. Wound healing is also an inflammatory reaction characterized by a cascade of neuro-regulatory, neuro-trophic, biochemical, immunological and functional disorders, microcirculatory and metabolic disorders [7]. The healing of the postoperative wound after flap surgery in patients with generalized periodontitis II, III degrees of severity also represents an inflammatory reaction with the appropriate ratio of the phases of necrotic and reparative processes. It is known that the intensity and duration of the inflammatory reaction during the healing of a myocardial infarction is determined by the form of the initial reactivity of the body and determines the complicated and uncomplicated consequences [15]. In the conducted study, we showed that with a different state of reactivity of the body, the nature of the inflammatory reaction in the postoperative period varies in intensity and duration, which determines different terms of clinical wound healing.

With normoreactivity of the organism, the wound healing times were normal (on the 9th day the state of the gums corresponded to «healthy»), with impaired (hyper- and hypo-) reactivity - they were delayed by 3-4 days compared to those with normoreactivity with the corresponding clinical the dynamics of the studied indices, which indicate the slow-down, sluggish nature of the inflammatory reaction in the postoperative period. Correction of delayed postoperative wound healing in patients with generalized periodontitis with reduction to values with normoreactivity is considered as a condition for optimizing wound healing after surgical treatment and further stabilization of the process in periodontal tissues.

CONCLUSIONS

Patients with generalized periodontitis accompanied by normoreactivity of the organism have normal terms of healing of a wound after the patch operation, which on the 9th day was characterized by a clinical condition of a mucous membrane of gums which corresponded to the concept "healthy" for 98% of patients. In patients with generalized periodontitis with hyperreactivity of the body there was a slowdown in wound healing for 3-4 days compared with that, which is typical for normoreactivity with the corresponding clinical dynamics of the studied indices, which showed prolonged intense inflammatory reaction in the postoperative period. In patients with generalized periodontitis with hyporeactivity of the body as well as with hyperreaction, there was a slowdown in wound healing for 3-4 days in comparison with normoreactivity with the corresponding clinical dynamics of the studied indices, which indicated a slow sluggish inflammatory response in the postoperative period.

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ORIGINAL ARTICLE

PECULIARITIES OF THE GUT MICROBIOTA IN PATIENTS WITH MIGRAINE COMPARING TO HEALTHY INDIVIDUALS

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ABSTRACT

The aim: Analyze the gut microbiome state in patients with migraine (M) and healthy individuals, to assess possible correlations between the detected changes in patients with migraine and the frequency, intensity of headaches, psycho-emotional state of the patients, and their quality of life.

Materials and methods: 100 objects were enrolled, divided into 2 groups: main – patients with M and control – healthy volunteers. Investigation of the intestinal microbiome was performed by chromato-mass spectrometry. For M patients the following scales were used: Visual Analogue Scale (VAS), Migraine Disability Assessment (MIDAS), Back Depression Inventory (BDI).

Results: In main group increased amount of *Alcaligenes spp* (p = 0.0061), *Clostridium coccoides* (p = 0.0021), *Clostridium propionicum* (p = 0.0287), *Eggerthella lenta* (p = 0.0138), *Pseudonocardia spp* (p = 0.0210), *Rhodococcus spp* (p = 0.0164), *Candida spp* (p = 0.0079), *Micromycetes spp* (campesterol) (p = 0.0011) were found. Patients with M had a raised amount of *Herpes simplex* (p = 0.0305) and endotoxin level (p = 0.0459). Differences in gut microorganisms in both groups were significant. In patients with M negative correlations were observed between *Alcaligenes spp* ammount and BDI score (r = -0.6226, p =0.007), VAS score (r = -0.489, p = 0.046), headache frequency (r = -0.487, p = 0.046); between the levels of *Clostridium coccoides* and MIDAS score (r =-0.51, p = 0.035), BDI score (r = -0.54, p = 0.025) and positive correlation between *Eggerthella lenta* level and VAS score (r =0.4830, p=0.049).

Conclusions: Correlations between changes of gut microbiome and M are promising for further research.

KEY WORDS: migraine, gut microbiota, depression, influence

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INTRODUCTION

Migraine is the second leading cause of headache and illness in young people, has a significant negative impact on the daily life of patients and interferes with their work, leisure, and social activities [1]. The gut-brain axis, which refers to the bidirectional biochemical signaling pathways that connects the gastrointestinal tract with the central nervous system, and vice versa, plays a significant role in the pathophysiology of migraine [2]. There's data on the significant prevalence of migraine in patients with certain gastrointestinal diseases, such as Helicobacter pylori infection, irritable bowel syndrome, and celiac disease [3], as well as the coexistence of migraine with conditions such as constipation and dyspepsia [4].

It's believed that the effects on the gut microbiome can be used to treat a number of diseases of the nervous system [5]. However, the issue concerning the role of microbiota in the pathogenesis of migraine and the effectiveness and safety of probiotics in the treatment of migraine patients requires further investigation [6]. At the same time, chronic stress and mental disorders, including depression and anxiety, contribute to the release of proinflammatory neurotransmitters, which is a prerequisite for the formation of chronic inflammation of the intestinal wall, which, in turn, has a potential negative impact on the gut microbiota [7]. Recent studies have shown significant progress in understanding the possible role of microbiota and their interaction with diet and lifestyle in patients with migraine [7]. It is known that changes in the microbiota can affect the normal absorption of nutrients, permeability of the intestinal wall, and immune function, disrupting the functioning of the gut-brain axis. This can affect the production of some important substances by gut microbiota such as calcitonin gene-related peptide (CGRP, an important mediator of migraines), IL-10 and serotonin [4].

THE AIM

The aim of the study was to analyze the gut microbiome state in patients with migraine and healthy individuals, to assess possible correlations between the detected changes in patients with migraine and the frequency, intensity of headaches, their psycho-emotional state, and quality of life.

MATERIALS AND METHODS

In total, 100 people aged from 18 to 55 years old (mean age 38.6 ± 8), among them 14.7% men, and 85.3% wom-

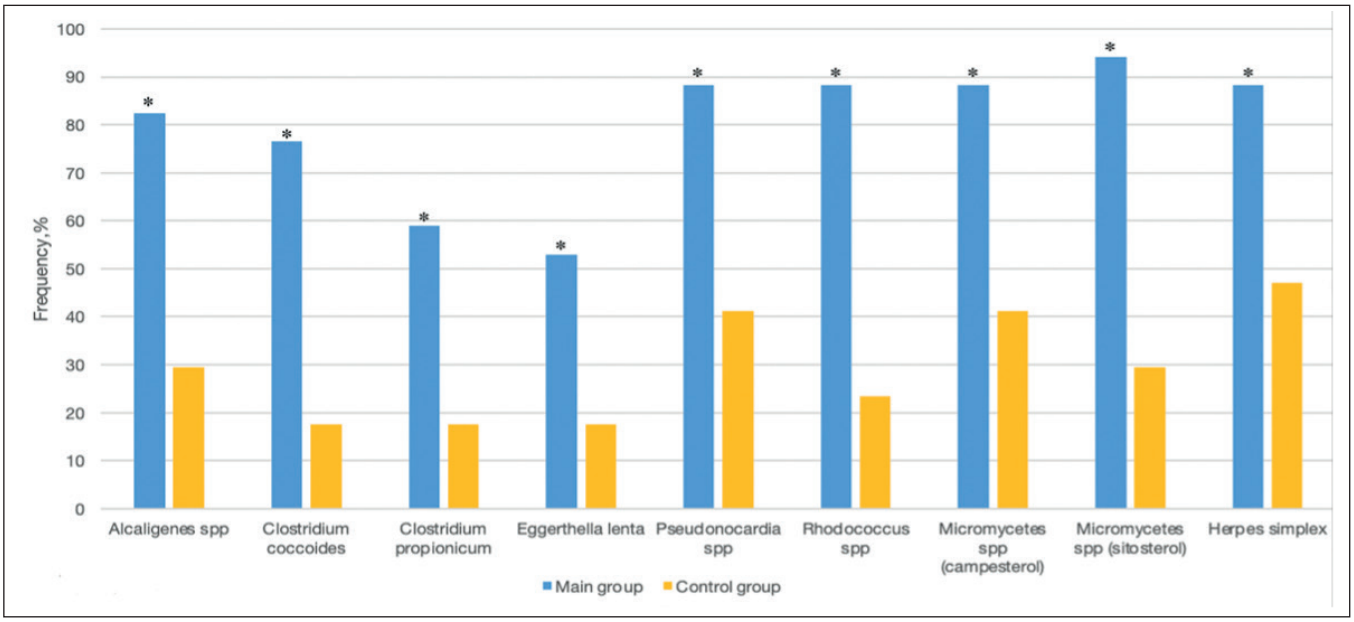

Fig. 1. Distribution of gut microbiota in both groups.
*p< 0,05

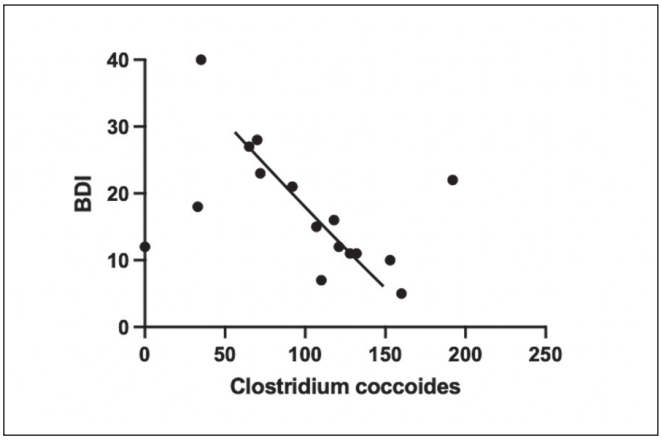

Fig. 2. The regression relationship between *Clostridium coccoides* level and BDI score in the patients with M.

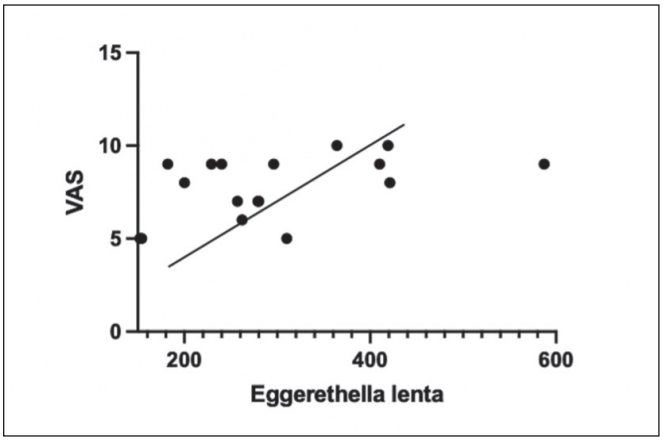

Fig. 3. The regression relationship between *Eggerthella lenta* level of and VAS score.

en were enrolled. The study didn't include patients under the age of 18 and pregnant women. Subjects were divided into 2 groups depending on the presence of migraine: the main - patients with chronic and episodic forms of migraine, the control - healthy individuals. Qualitative and quantitative assessment of pain severity was performed using a visual-analog scale (VAS), psycho-emotional status was assessed using Hamilton's anxiety (HARS) and Beck's Depression Inventory (BDI). To establish the degree of social maladaptation, the Migraine Disability Assessment Scale (MIDAS) was used. The investigation of gut microbiota was performed by chromato-mass spectrometry, which determined not only the qualitative, quantitative composition of resident and transient microorganisms, but also microscopic fungi, chemical load of viruses.Statistical analysis of data obtained was performed using GraphPad Prism version 9.3.0. Student's t-test (t) was applied for evaluating credibility between mean quantitative positions of two samples. Proportions were compared using $\chi 2$. Relationships between different indicators were assessed using the Pearson's correlation coefficient (r) according to statistical distribution.

RESULTS

In the main and control groups there was no significant difference in content of *Actinomycetes (Actinomyces spp, Actinomyces viscosus)* ($p = 0.46$; $p = 0.45$). For *Alcaligenes spp*, which belong to resident microorganisms, substantial changes in their quantitative composition ($p = 0.006$) in patients with migraine were found. Also in the main group a notable increase in the quantitative composition of *Clostridium coccoides* ($p = 0.002$) and *Clostridium propionicum* ($p = 0.028$) was detected. While no significant differences between groups were found in the content of *Clostridium perfringens* ($p = 0.76$), *Clostridium ramosum* ($p = 0.12$), and *Clostridium tetani* ($p = 0.46$). A significant increase in the level of *Eggerthella lenta* ($p = 0.013$) was revealed

in the main group. Among resident microorganisms, the level of *Pseudonocardia spp* (p = 0.02) and *Rhodococcus spp* (p = 0.016) was increased in patients with migraine, compared to the control group. Regarding other microorganisms, no significant changes in their quantitative composition in the main group in comparison with the control were found. For the transient group, including *Bacillus cereus, Bacteroides fragilis, Bacteroides hypermegas, Campylobacter mucosalis, Clostridium difficile, Enterococcus spp, Flavobacterium spp, Helicobacter pylori, Kingella spp, Acinetobacter spp,* and *Peptostreobipostecoccinus E coli*, no qualitative changes were found in either the main or control groups. The number of microscopic fungi such as *Candida spp* (p = 0.007), *Micromycetes spp* (campesterol) (p = 0.001), and *Micromycetes spp* (sitosterol) (p= 0,001) were increased in patients with migraine compared to the control group. The study of the chemical load of viruses in a comparative aspect showed a significant increase in the number of *Herpes simplex* in patients with migraine (*Herpes simplex*) (p = 0.03). Changes in other viruses were not significant. In the main group, a valid increase in the amount of endotoxin (p = 0.04) was found.

Patients with migraine had a significantly higher frequency of elevated *Alcaligenes spp* ($\chi2 = 9.66$, p = 0.001), *Clostridium coccoides* ($\chi2 = 11.81$, p = 0.006), *Clostridium propionicum* ($\chi2 = 6.1$, p = 0.013), *Eggerthella lenta* ($\chi2 = 4.63$, p = 0.03), *Pseudonocardia spp* ($\chi2 = 8.24$, p = 0.004), *Rhodococcus spp* ($\chi2 = 14.44$, p = 0.0001), *Micromycetes spp* (campesterol) $\chi2 = 8.24$, p = 0.004) and *Micromycetes spp* (sitosterol) ($\chi2 = 15.7$, p = 0.0001), and *Herpes simplex* ($\chi2 = 6.58$, p = 0.01) compared to the control group (Fig.1).

In the main group, a negative correlation was found between the level of *Alcaligenes spp* and the score on the BDI score, HARS score (respectively: r = -0.62, p = 0.007; r = -0.51, p = 0.03),the frequency of attacks (r = -0.49, p = 0.04) and the VAS score (r = -0.48, p = 0.04). Significant negative correlation was found between elevated level of *Clostridium coccoides* and MIDAS score (r = -0.51, p = 0.03), BDI score (r = -0.54, p = 0.02) in the patients with migraine. (Fig.2) .

At the same time, in the main group a positive correlation was detected between *Eggerthella lenta* level of and VAS score (r = 0.48, p = 0.049) (Fig.3) .

In the main group negative correlations were found between slightly increased content of endotoxin in the main group, the BDI score, HARS score (respectively: r = -0.69, p = 0.001; r = -0.56, p = 0.02) the frequency of attacks (r = -0.55, p = 0.023), on the VAS score (r = -0.53, p = 0.028), as well as MIDAS score (r = -0.56, p = 0.02).

DISCUSSION

Among actinomycetes (*Actinomyces spp, Actinomyces viscosus*), which are common inhabitants of the gastrointestinal tract and oral cavity, no reliable changes were found in the main and control groups. For *Alcaligenes spp*, which belong to resident microorganisms (normally inhabit gastrointestinal tract), significant changes were detected in the quantitative composition in the main group compared to the control one. *Alcaligenes* produce antibiotics and original antibacterial components that disrupt the growth of a wide range of bacteria, as well as initiate growth of B-lymphocytes and Alcaligenes-specific antibodies, to create their own "cloak" coating [8]. We found an elevated incidence of the increased number of these microorganisms in the main group and their negative relationship with the BDI and HARS score, the frequency of migraine headaches, and the VAS score. Thus, the higher amount of these microorganisms corresponds to the lower level of depression and anxiety, and the less severity and frequency of headache in the patients of the main group. *Clostridia* are normally present in the human intestine, on the skin, mucous membranes of the oral cavity, in the genital system, and in the respiratory tract [9]. Many clostridia are pathogens of dangerous diseases (*C. botulinum, C. tetani, C. perfringens*), but it should be noted that due to the regulatory effects of microbial biofilms and the immune system, these diseases are not caused. The peculiarity of the negative impact of clostridia in the case of their excessive growth is the production of powerful bacterial exotoxins, as well as a number of proteolytic enzymes, which lead to local tissue damage [9]. The microbiome study showed a significant increase in the quantitative composition of *Clostridium coccoides* and *Clostridium propionicum* in migraine patients, along with a rise in their frequency in migraine patients comparing to those from the control group. *Clostridium propionicum* produces propionic acid as the main product of fermentation. *Propiobacteria* are *gram-positive*, *non-spore-forming*, *anaerobic* and *rod bacteria*. *Propiobacteria* are involved in the synthesis of vitamin B12, probiotics and propionic acid [10]. Regarding the increased level of *Clostridium coccoides*, they have an important prebiotic effect. Their level is directly related to the improvement of intestinal function, and they can enhance the growth of lactobacilli and have an inhibitory effect on a number of harmful microorganisms [11]. We found a negative correlation between elevated *Clostridium coccoides*, *MIDAS*, and *BDI scores* in migraine patients, indicating their potential positive impact on depression severity and quality of life in migraine patients. A significant increase in *Eggerthella lenta* in the main group may indicate inflammatory changes in the gastrointestinal tract in patients with migraine [12]. At the same time, the elevated level of these microorganisms was associated with increased pain severity according to *VAS score*.

In the patients with migraine, compared to the control group, the number of microscopic fungi such as *Candida spp, Micromycetes spp* (campasterol, sitosterol) was increased. *Candida* is a conditional pathogen that lives on mucous membranes. There are about 140 species of *Candida* (most of which are pathogenic), which under certain conditions lead to candidiasis and weaken the immune system. It is established that colonization of the gastrointestinal tract by *Candida* fungi is asymptomatic. Assessing the chemical load of viruses (*Herpes simplex*, *Epstein-Barr virus and cytomegalovirus*) in a comparative

aspect, we found a potential increase in the number of *Herpes simplex* in patients with migraine. In the main group, slightly incresed amount of endotoxin was found. *Endotoxin*, or *bacterial lipopolysaccharide* (LPS), is considered to be the most potent mediator of microbial origin involved in the pathogenesis of sepsis and septic shock. Small doses of LPS in a limited tissue space help the host to organize effective antimicrobial protection, and facilitate the removal of pathogens to the environment. At the same time, the sudden release of large amounts of LPS, on the contrary, has a negative impact on the human body as far as it triggers uncontrolled and the life-threatening release of numerous inflammatory mediators and procoagulants into the systemic circulation [13]. The negative correlations between slightly elevated total endotoxin level and VAS, MIDAS, BDI, HARS score, frequency of migraine attacks, indicate its possible positive effects on the intensity, frequency of pain, quality of life of migraine patients, and their psycho-emotional state.

CONCLUSIONS

1. The significant difference in the composition of the gut microbiota in patients with migraine comparing to healthy individuals was found.
2. In patients with migraine a remarkable influence of gut microbiome changes on the characteristics of headache and indicators of psycho-emotional status was established.
3. The revealed peculiarities of gut microbiome changes in patients with migraines need further clarification in order to identify the possible role of the gut microbiome in the pathogenesis, clinical picture, and therapy of migraine, and is a promising area of further scientific research.

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ORIGINAL ARTICLE

GONADOTROPIN AND SEX STEROID HORMONES IN MALES WITH POST COVID-19 INFECTION

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ABSTRACT

The aim: To understand the effects of COVID-19 infection on gonadotropins and sex steroid hormones in males.

Materials and methods: This is a cohort study conducted in fifty males, who had been previously infected with COVID-19 with normal hormonal profile. Fifty Iraqi males were attending the male clinic at Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies, diagnosed with standard methods. The assessment of serum hormonal levels including (FSH, LH, Prolactin and Testosterone) was done 3 times: 1st time after one-month post recovery after COVID-19, 2nd time after 2 months post recovery and 3rd time - after 3 months post recovery.

Results: There was no significant change in the mean level of serum FSH during the first, second and third months ($p = 0.630$). LH serum level was highly significantly reduced during follow up ($p < 0.001$). Serum prolactin level, reduced significantly during follow up ($p < 0.001$). Serum testosterone level was the lowest in the first month and increased during the second month and then during the third month in a highly significant manner ($p < 0.001$).

Conclusions: Sub-clinical hypogonadism may be suspected as a consequence of COVID-19 infection in males as its first presentation characterized by increased LH & decreased testosterone production.

KEY WORDS: COVID-19, hypogonadism, testosterone, gonadotropin

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INTRODUCTION

At present day a highly contagious viral infection that spreads very quickly globally on Feb. 2020, was announced as the new viral infection, known as COVID-19. On March 2020 the WHO declared COVID-19 as a global pandemic [1]. SARS-CoV-2 surface spike (S) viral proteins often bind to ACE2 and TMPRSS2, and through this, the virus invades the host [2-3]. Recent studies suggest that the symptoms in COVID-19 patients include problems in brain and testes [4-5]. Many pathogenic signs were seen owing to the neuro invasive trait of SARS-CoV-2, as seen in COVID-19 [6-8]. SARS-CoV-2 has been shown to breach the blood-brain barrier (BBB) and infect ACE2 expressing neurons and glial cells, leading to neuropathology in the brain regions, including the hypothalamus, which regulates several processes including temperature control and hormonal balancing [9-10]. As for SARS-CoV-2-mediated dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis, nothing is known about the possibility of SARS-CoV-2-mediated effects on the aberrant HPA axis [11]. The HPG feedback loop links the brain and testes through gonadotropin and testosterone [12-13].

THE AIM

The aim of this work was to understand the effects of COVID-19 infection on gonadotropins and sex steroid hormones in males.

MATERIALS AND METHODS

Fifty Iraqi males were selected from those who were attending the male clinic at Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies. With informed consent all patients provided a full medical history and underwent full physical examination. The study group was observed and was tested 3 times: 1st time – after 30 days post-recovery, 2nd time - 60 days and 3rd time 90 days post COVID-19, respectively. Inclusion criteria: Age ≥18 and ≤50 years; normal hormonal profile (FSH, LH, Prolactin & Testosterone) before COVID-19 infection. Exclusion criteria: age <18 and >50 years, abnormal hormonal profile before COVID-19 infection. Blood samples were drawn from each subject (5ml) for FSH, LH, testosterone, prolactin assays. Hormonal analysis was performed by using mini VIDAS apparatus (VIDAS), through an enzyme linked fluorescent assay (ELFA) technique. The kits were purchased from Bio Merieux and labeled VIDAS®FSH, VIDAS®LH, VIDAS®TESTOSTERONE and VIDAS®PROLACTIN. The normal range for the hormones was (2.1–18.6) μIU/ml for FSH, (1.7–11.2) μ IU/ml for LH, (3.6-16.3) ng/ml for prolactin and (262-870) ng/ml for testosterone.

RESULTS

The current study included 50 fertile males with COVID-19 with a mean age of 33.66 ±6.00 years and an age range of 24 to 48 years. The frequency distribution of patients with COVID-19

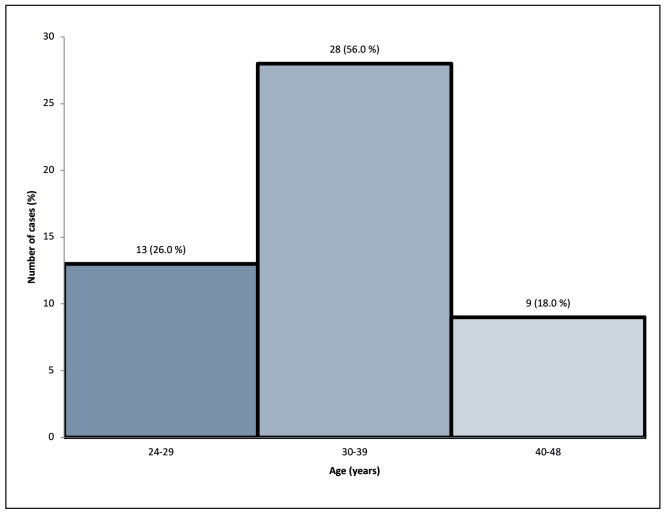

Fig. 1. Histogram showing the frequency distribution of patients according to age

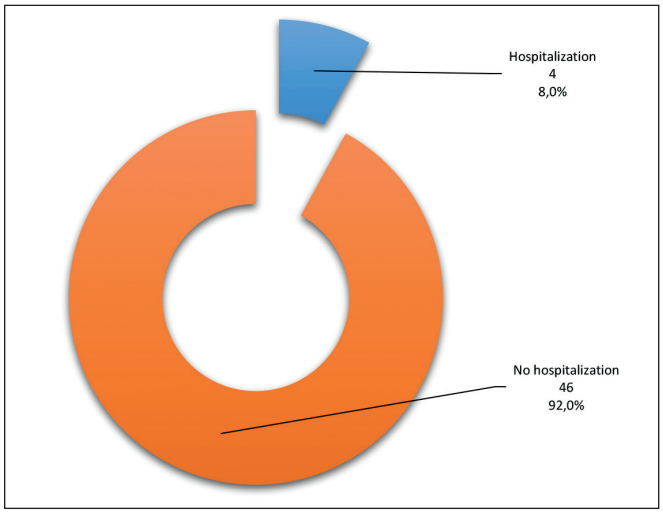

Fig. 2. Pie chart showing the frequency distribution of patients with COVID-19 according to hospitalization

Table I. Quantitative assessment of serum hormonal levels in patients with COVID-19 during three months post-recovery follow up

Characteristic	First month	Second month	Third month	p
FSH				
Mean ±SD	6.97 ±4.18 A	7.15 ±3.79 A	6.90 ±2.87 A	0.630 Pi NS
Range	2.5 -23.2	2.3 -21.4	3.5 -19.5	
LH				
Mean ±SD	8.42 ±4.55 A	7.79 ±4.17 B	6.18 ±2.88 C	< 0.001 Pi HS
Range	2.5 -21.3	2.2-17.8	3.2 -14.2	
Prolactin				
Mean ±SD	9.60 ±3.54 A	10.04 ±2.36 A	8.01 ±2.30 B	< 0.001 Pi HS
Range	4.5 -24.4	4.6 -15.6	4.5 -14.2	
Testosterone				
Mean ±SD	162.95 ±61.39 C	198.19 ±69.43 B	331.43 ±143.83 A	< 0.001 Pi HS
Range	35.33 -255.37	52.4 -310.4	80.45-730.4	

SD: standard deviation; IQR: inter-quartile range; Pi: Pillai's Trace repeated measure test; HS: highly significant at p ≤ 0.01; capital letters (A, B and C) were used in order to show the level of significance following post hoc multiple comparison LSD test so that similar letters indicant no significant difference while different letters indicate significant difference

according to age is shown in figure (1) in which it is observed that 13 (26.0 %) males were between 24 and 29 years, 28 (56.0 %) of them were between 30 and 39 y.o. and 9 (18.0 %) were between 40 and 48 years. The frequency distribution of patients with COVID-19 according to hospitalization is shown in figure (2) the proportion of fertile men with COVID-19 with hospital admission was 4 out of 50, accounting to 8.0 %.

The quantitative assessment of serum hormonal levels in patients with COVID-19 during three months post-recovery follow up is shown in table I. There was no significant change in the mean level of serum FSH during the first, second and third months (p=0.630). Regarding serum LH level, it was highest in the first month and was reducing during the second month and then during the third month in a highly significant manner (p<0.001). Regarding serum prolactin level, it was the highest in the first and the second months, then got significantly reduced during the third month (p<0.001). Regarding serum testosterone level, it was the lowest in the first month and got increased during the second month and then during the third month in a highly significant manner (p<0.001). The qualitative assessment of serum hormonal levels in patients with COVID-19 during three months post-recovery follow up is shown in table II.

DISCUSSION

In the current study the distribution of COVID-19 among age groups is highly presented in 30-39 years old 4th

Table II. Qualitative assessment of serum hormonal levels in patients with COVID-19 during three months post-recovery follow up

Characteristic	First month		Second month		Third month	
	n	%	n	%	n	%
FSH						
Low	0	0	0	0	0	0
Normal	49	98	49	98	49	98
High	1	2	1	2	1	2
LH						
Low	0	0	0	0	0	0
Normal	39	78	42	84	46	92
High	11	22	8	16	4	8
Prolactin						
Low	0	0	0	0	0	0
Normal	49	98	50	100	50	100
High	0	0	0	0	0	0
Testosterone						
Low	50	100	40	80	12	24
Normal	0	0	10	20	38	76
High	0	0	0	0	0	0

decade of life with a percentage 56% of cases. While the percentage of cases in the age group between 24-29 years old 3rd decade of life is 26%; moreover, the percentage becomes lower in the 5th decade of life (the percentage was down to only 18% in subjects of 40-48 years old figure (1)). On the other hand, a study conducted in Japan shows increasing risk to have COVID-19 in older age, with the highest risk is likely among male adults aged between 50-69 years old and less risk in lower age groups with very infrequent in males aged 0-19 years old [14]. Only 8% of the cases for this study required hospitalization to manage their COVID-19 infection, while the rest of the cases didn't require admission to hospitals with no relation between hospitalization and age groups as all hospitalized cases belong to different age groups as seen in figure (2). However, in [15] hospitalization rates increased with age and were the highest among older adults; the majority of hospitalized patients had underlying conditions. On the other hand, another study conducted in France to compare between COVID-19 patient in different time frames first period in March-April 2020 and the second period in June-August 2020 shows significantly younger patients in the second period with mainly males rather than females. Also, that study showed the need for hospitalization was down to 10% in the second period by contrast their viral load was higher in the second period in comparison to the first period [16].

By performing quantitative assessment of serum hormonal levels (FSH, LH, Prolactin and Testosterone) in patients with COVID-19 during three months of post-recovery follow up is shown in table I. There was no significant change in the level of serum FSH during the first, second and third months. Regarding serum LH level, it was highest in the first month and got reduced during the second month and then during the third month in a highly significant manner. Regarding serum prolactin level, it was highest in the first month and the second months and got reduced during the third month although, this change is statistically significant but clinically isn't as serum prolactin levels during the 3 months period for all cases remained within normal physiological levels. Regarding serum testosterone level, it was lowest in the first month and got increased during the second month and then during the third month in a highly significant manner. In a study done by [17], COVID-19 patients had significantly higher serum LH. Although there was no statistical difference in serum T or FSH, the ratios of T: LH and FSH: LH were decreased significantly in the COVID-19. Also, another study found similar results to ours, in a cohort study of 45 male COVID-19 patients, 68.6% and 48.6% of patients had low T and dihydrotestosterone levels, respectively [18]. In comparison to patients in remission, 31 males in critical care (or deceased) had higher LH, lower total T and calculated free T which suggests hypogonadism in severely ill patients [19]. By performing a qualitative assessment to serum hormonal levels as seen in table II, serum FSH and Prolactin levels remain within normal physiological levels during the 3 months period post recovery from COVID-19; whereas serum Testosterone level was lowest in the 1st month post recovery and continues to improve over the 2nd and 3rd month in highly significant manner, while serum LH level was highest in the 1st month post recovery and continues to drop down during the 2nd and 3rd in a highly significant manner. These results are in agreement with [20], COVID-19 patients had significantly higher serum LH levels but decreased testosterone/LH and FSH levels than healthy men, suggesting potential hypogonadism. Taken together, patients

with COVID-19 have been found to present a reduced testosterone/LH ratio, indicating possible subclinical damage to male gonadal function. Additionally, activation of the HPGa and subsequent alterations in hormone concentrations play a critical role in poor sperm quality.

CONCLUSIONS

From this study it was concluded that the COVID-19 infection led to decrease in testosterone, increase in LH production and no significant effect on FSH or prolactin levels. COVID-19 infection may lead to sub-clinical hypogonadism in males as it clinically characterized by increase in LH production with or without decrease in testosterone levels because increment in LH production may compensate testosterone for a period of time.

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ORIGINAL ARTICLE

COMPLICATIONS OF NEPHROTIC SYNDROME IN CHILDREN

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ABSTRACT

The aim: Study was conducted to determine NS-related complications and drug-related complications.

Materials and methods: A descriptive analytics cross section (case series study) was carried out in 109 children with NS diagnosed and followed up in the nephrology consultation clinic at Child Welfare Teaching Hospital in Baghdad Medical City.

Results: Most children have primary NS 88% and 12% Secondary NS. Nephrotic syndrome related complication: higher rate of infections and hypertension complications 76% and 48.6% respectively recorded in children with NS. 60.3% of infections in children with Frequent relapses and 25% in SSNS with statistically significant (p value0.018). Children with NS; have higher rate of UTI and Pneumonia.64% of Hypertension recorded in children with frequent relapses while 13.2% identified in children with secondary NS, with statistically non-significant (p value 0.063) 24% and 9% of patients have AKI &CKD respectively. Drug related complication: Children used steroid; have 34.5% Cushing face,30%gastritis,6.5% cataract,19.7% obesity and22.4% short stature. Children used cyclosporine; have33% hirsutism,9.5% nephrotoxicity and4.7% gingival hyperplasia, 27% vomiting, 22% alopecia and 11%Bone marrow suppression side effect with Cyclophosphamide. Patient used mycophenolate drugs, have 15.5% GIT problem and 7.7% BM suppression.

Conclusions: NS is a chronic disease with a lot of complications like infections, AKI &CKD and hypertension complications. UTI and pneumonia are most common infection encountered in our patients. Many of the complications of children with NS can be attributed to immunosuppressant therapy.

KEY WORDS: Nephrotic syndrome, children, complications

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INTRODUCTION

Nephrotic syndrome (NS) is a common and important chronic renal disease in children. NS is characterized by massive proteinuria, hyopoalbuminemia, and edema; although additional clinical features such as hyperlipidemia are also usually present [1]. Complications of NS are divided into two categories: disease-associated and drug-related complications. Disease-associated complications include infections (e.g., peritonitis, sepsis, cellulitis, and chicken pox), Thromboembolism (e.g., venous thromboembolism and pulmonary embolism), hypovolemic crisis (e.g., abdominal pain, tachycardia, and hypotension), cardiovascular problems (e.g., hyperlipidemia), acute renal failure, anemia, and others (e.g., hypothyroidism, hypocalcaemia, bone disease, and intussusception). The majority of children with MCNSwho respond to treatment with corticosteroids or cytotoxic agents' havesmaller and milder complications than those with steroid-resistant NS. Corticosteroids, alkylating agents, cyclosporine A, and mycophenolate mofetil have often been used to treat NS, and these drugs have treatment related complications [2]. Prolonged high dose steroid therapy may be associated with significant side effects. Steroids result in an increased appetite, adrenal suppression, impaired growth, gastritis, salt and water retention, hypertension, striae, cataracts, myopathy, metabolic alkalosis, bone demineralization, aseptic bone necrosis, diabetes, decreased immunity and subsequent increased risk of infection. In addition, behavioral and psychological consequences are common, including anxiety, depression and aggressive behavior; children with frequently relapsing and steroid dependent NS are at increased risk of these adverse effects [3-4].

THE AIM

Study was conducted to determine NS-related complications and drug-related complications.

MATERIALS AND METHODS

A Descriptive analytics cross section (case series study) has been carried out on 109 children (71 males and 38 females) with N.S who were diagnosed and followed up in the nephrology consultation clinic are going to be included in the study, from the 1st of November 2019 to the end of November 2020. Their ages ranged from 1-15 years, some of the patients admitted to nephrology ward at Child welfare Teaching Hospital in Baghdad Medical City.

INCLUSION CRITERIA

All Patients with nephrotic syndrome: Primary and secondary NS

EXCLUSION CRITERIA ARE

- Age younger than 1year or older than 14 years.

NEPHROTIC SYNDROME

Diagnosis of NS requires the presence of edema, massive proteinuria (>40 mg/m2/hr or a urine protein/creatinine ratio >2.0 mg/mg), and hypoalbunemia (<2.5 g/dl) [1].

Primary Nephrotic syndrome is defined as Nephrotic syndrome in the absence of systemic disease.

Secondary nephrotic syndrome is defined as nephrotic syndrome associated with systemic diseases or is secondary to another process that causes glomerular injury [5].

Definitions regarding the response to corticosteroid therapy are as:

Follows: 3 Remission: Urinary protein to creatinine ratio < 0.2 or < 1 + protein in urine dipstick testing for 3 consecutive days.

Relapse: is characterized as the urinary protein / creatinine ratio of more than 2.0 mg / mg or the urinary albumin dipstick ≥3 + for three consecutive days.

Steroid-Sensitive Nephrotic Syndrome (SSNS): remission of patients within the first four weeks of treatment with steroids.

Steroid-Resistant Nephrotic Syndrome (SRNS): patients who have no remission after 8 weeks of treatment with corticosteroids.

Steroid dependence: 2 consecutive relapses during steroid therapy or within 2 weeks of discontinuation of steroid taper therapy.

FREQUENT RELAPSE

Tow or more relapses within 6 months of initial response, or 4 or more relapses within 12 months.The medical records of all children with NS aged 1-15years who were referred to the hospital were reviewed. The following Information regarding patients (age at onset NS, clinical presentation, complications, number of nephrotic relapses, response to steroid, immunosuppressive drug, lab and renal biopsy findings,) were taken from patients records and direct interview with patient's family are included in the study, Cases were selected in random fashion. A special questionnaire was designed for the purpose of the study the following information was obtained: Identity name, age, sex and residence past medical history: age at diagnosis of Nephrotic syndrome, NO, of relapse, frequent relapse, infrequent relapse.

FAMILY HISTORY

Parents or sibling who have renal disease.

DRUGS HISTORY

Drug Corticosteroids, alkylating agents, cyclosporine, mycophenolate mofetil, and rituximab have often been used to treat NS.

Response to steroid therapy, Steroid resistant, Steroid dependence, and side effect of drug

PHYSICAL EXAMINATION

All patients underwent general and systemic examination, vital signs were measured (temperature, respiratory rate, heart rate) and anthropometric measurements include (WT and HT or length) were assessed and applied to appropriate charts according to CDC/WHO normalized references, the Weight-for-height Z score, height for-age Z score and weight-for-age Z score were estimated. Blood pressure measurement (applied to the blood pressure levels by age and height percentile). Investigations were done for patients according to their clinical manifestation as complete blood count, blood culture, CXR, microscopically urine examination and culture, renal function test, urine dipsticks for albumin, 24h urine collection for albumin, serum albumin, serum electrolyte, lipid profile and other. GFR was calculated according to the Original Schwartz Equation [6].

GFR (ml/min/1.73 m2) = K × (height in cm (or length))/ (Serum Cr (mg/dl))

K =

0.33-Low-birth-weight babies (<2.5 kg birth weight) in first year of life

0.45-Term, normal infants during first year of life

0.55-Children and adolescent girls

0.70-Adolescent boys

An informed consent was obtained from the parents for all children recruited in the study.

STATISTICAL ANALYSIS

Data were analyzed using SPSS software V.26, expressed as a mean ± Standard Deviation, the Chi-Square test was used to compare proportions. For the quantitative comparison of variables of patients included in the study, the t-test and one-way analysis of variance (ANOVA) was used, for all tests p-value of < o.o5 was considered as statistically significant.

RESULTS AND DISCUSSION

A total of 109 children with NS were included in the study, their ages ranged from 2-14 years, Mean Age was 8.06 ±3.85, 96 children with primary NS and 13 Secondary NS patients were included. There is no significant difference concerning sex and age distribution between patients with primary NS and Secondary NS groups.

Despite investigations, the exact cause of childhood nephrotic syndrome remains unknown in a large number of cases. These are labeled as idiopathic or primary Nephrotic syndrome. In this Descriptive analytics cross section (case series study) Observed thefrequency of NS was slightly predominant in males than females and a male to female ratio of 1.8:1 This is in agreement with the results of other study carried out in Iraq [7]. Renal biopsy

Table I. Complication in relation to frequency of relapse

Complication	Primary NS	N0.	(%)	Secondary NS N0 (%)	Total N0 (%)	P Value*
	SSNS (Infrequent Relapse)	SDNS (Frequent Relapse)	SRNS			
Infection	21(25)	11(13.3)	39(47)	12(14)	83(76)	0.018
Pneumonia	2 (8)	3(12)	15(60)	5 (20)	25(30)	0.017
UTI	16(24)	8(1.9)	34(50.5)	9 (13.4)	67(80.5)	0.023
Peritonitis	1 (10%)	2 (20)	5(60)	1 (10%)	10(12)	0.464
Cellulitis	1(8.3)	2(16.)	8(66.7)	1 (8.3)	12(14.4)	0.259
sinusitis	1(33.3)	0(0)	2(66.7)	0 (0)	3(3.6)	0.375
Sepsis	0 (0%)	0 (0)	4 (50)	4 (50)	8(9.6)	0.003
Meningitis	0 (0%)	1(25)	1 (25)	2 (50)	4(4.8)	0.100
Abscess	0 (0%)	1(50)	1(50)	0 (0)	2(2.4)	0.575
other infection	2(20)	1(10)	7(70)	0 (0)	10(12)	0.242
ARF CKD	5(19.2) 1(10.0)	4(15.4) 1(10.0)	13(50) 8(80)	4(15.4) 0(0)	26(24) 10 (9)	0.042**
pleural effusion	2(12.6)	3(18.8)	5(31.3)	6(37.5)	16(14.7)	0.004
pneumothorax	1(50)	0(0)	1(50)	0(0)	2(1.8)	0.575
thrombosis	0(0)	1(20)	3(60)	1(20)	5(4.6)	0.599
Seizure	4(26.7)	1(6.7)	7(46.7)	3(20.0)	15(13.8)	0.612
Hypertension	12(22.7)	6(11.3)	28(52.8)	7(13.2)	53(48.6)	0.05
Hypocalcaemia Vit D deficiency Hypothyroidism Anemia	4(26.7) 2(33.3) 2(66.6) 5(22.7)	0(0) 0(0) 1(33.3) 4(18.2)	11(73.3) 2 (33.3) 0 (0) 9 (40.9)	0(0) 2(33.3) 0 (0) 4(18.2)	15(13.8) 6(5.5) 3(2.8) 22(20)	0.022 0.261 0.464 0.609
Death	0(0)	0(0)	5(100)	0(0)	5(4.6)	0.114

usually is not performed in all patients with NS because most of them are MCD that respond to corticosteroids therapy [8].In this study, the renal biopsy done for 33% of cases, 75% of the histopathological result classified as primary nephrotic syndrome and 25% were diagnosed as secondary NS(SLE, IGA, Post-Infectious GN and etc). In the present study, infections were found in (76%) of patients with NS, this is in agreement with other study carried out by Alwadhi RK from New Delhi, India 83.8% [9]. The present study shows that 60% and 75% of patients with primary and secondary nephrotic syndrome respectively had urinary tract infection, thisis in agreement with other study carried out by Nahla I. Al-Gabban et.al from Iraq which was recorded in 59.20% [10]. Also observation of high prevalence of UTI among nephrotic syndrome patients have been reported by other study 66.7% of UTI has recently been documented from Africa [11] Similar high prevalence of UTI has also been reported in study from India [9], while in other studies it was found that UTI was relatively uncommon in These variations probably reflect geographical or socioeconomic heterogeneity of patient populations [12].

Pneumonia was the second commonest infection in our study 30%. Studies from India and other south East Asian countries showed high prevalence of Pneumonia [13-14]. This may be the reflection of overall increased prevalence of respiratory infections in the community over the last years. Mechanical pressure on lungs due to massive ascites and pleural effusions leading to stasis of lung fluids may be also contributing to the high frequency of pneumonia in these children.4 In the current study the incidence of peritonitis was found 12 % of casesand cellulitis 12 % of cases while peritonitis was ranged between 1.4% and 16% of children with NS in other studies [15-16] and this wide range could be related characteristic of studied group, our study was havefrequent relapses (steroid dependent or steroid resistance Nephrotic syndrome), while other study carried out by Krishnan C et al the frequency of cellulitis has decreased from 27.7% reported in previous study to 5% ,therelatively better vaccination coverage against pneumococci and betterliving conditions may be contributing to the low incidence of peritonitisand cellulitis in these series studies40. Septicemia is accounted 9.6% of cases in our study, similar to study of Chang-Chingwei et al [14].

The current study showed the rate severe gastroenteritis 7% while otherstudy carried in Egypt found the high incidence of gastroenteritis 25% of cases due to admission of

Table II. Complications of medication used in treatment NS

Parameters	NO.	Percent %
1-Steroid related Complications	60	56
Gastritis	32	30
Cushing face	37	34.5
Osteoporosis	3	2.8
Cataract glaucoma	7	6.5
Hirsutism	13	12
Obesity	21	19.6
Short stature*	24	22.4
2- Cyclophosphamide related	5	27
Vomiting	5	27
Alopecia	4	22
Bone marrow suppression	2	11
Hemorrhagic cystitis	0	0
3- Calcineurin inhibitor		
Related complication	15	35.7
Elevate renal indices	4	9.5
Neurotoxicity (Tremor)	1	2.3
Gingival hyperplasia	2	4.7
Cyclosporine hirsutism	14	33
Hyperglycemia	1	2.3
MMF related complications	4	15.5
GIT problem (diarrhea)	4	15.5
Bone marrow suppression	2	7.7
Rituximab related complications	1	50
Genital herpetic lesion	1	50

cases during summer with high rate of gastroenteritis [16]. Nephrotic syndrome documented to have high infection rate, the increased risk of infection is multifactorial and in part is due to the immuno dysregulation. Corticosteroid virtually affects all immune cells, and they antagonize macrophage differentiation as well as suppressmacrophage production of interleukin 1, interleukin 6, tumor necrosis factor, and the proinflammatory prostaglandins and leukotrienes. Glucocorticoids also suppress neutrophil adhesion. The duration of therapy is important but the exact doses and duration that substantially change thebenefit-risk equation for corticosteroids likely varies by individual and Their underlying risk for infection [17]. The current study showed the incidence of AKI according to the Pediatric .RIFLE definition of AKI has been found 24 % of cases, dialysis done for five cases while other on conservative management. This is in agreement with other study carried out by M. Sharma et al. [18], also the study showed the incidence of CKD in children with Primary Nephrotic syndrome; was 10.3%, which more common in SRNS patients, this is in agreement with the study carried out by Cho, H.Y. et al. that showed 9.8% of patients CRF and 21.7% had ESRD during period of the follow-up [19] patients with SRNS have a good prognosis if remission of proteinuriaachieved by medications other than steroids. Failure to respond to treatment causes kidney insufficiency and predictors of poor outcome and progression to ESKD [19] the Current study showed the incidence of HT in children with Nephrotic syndrome; 16%, more common in SRNS. This is in agreement with otherstudy carried out by Nahla I. Al-Gabban et al. from Iraq which was recorded 40% [10] also in agreement with other study carried by Ibrahim F. Shatat et al. [20] but it disagree with other studies carried in Iran which reported only 15.6% developed hypertension [21]. In our study, the frequency of thrombosis was 5% this is in agreement with another study reported that about 2% to 5% of children with Nephrotic syndrome suffer thromboembolism complications such as cerebral venous thrombosis CVT [22], while the study of Brice et al. on patients younger than 21 year with nephrotic syndrome showed that the incidence of cerebral thromboembolism was 9.2%, 55 Seizure occurred in 13.8% of our patient, 40% had hypertensionencephalopathy and 13.5% had cerebral thromboembolism similar to study carried in Qazvin, Iran [23]. In the present study 20% of cases had anemia and both normocytic and microcytic pictures were

seen. Iron deficiency anemia in Nephrotic syndrome is due to loss of transfer in the urine. In the present study hypocalcaemia and Vitamin D deficiency have been reported in 13.8% and 5.5% of NS case respectively. This agreement with result carried by Kersh, Mahmoud El, et al. reported Children with NS are at risk of vitamin D deficiency and hypocalcaemia [24].

Disturbances in calcium and vitamin D metabolism are not infrequent in nephrotic children and these abnormalities can occur with normal glomerular filtration rate GFR. These disturbances are mainly due to the loss of vitamin D metabolites, vitamin D binding protein DBP and minerals as calcium and phosphorus in urine, as well as steroid therapy .Hypocalcaemia is generally due to hypoalbunemia and a low protein bound fraction of serum calcium but can also be associated with a low level of ionized calcium [24] Our analysis shows that defects in thyroid function occurs in patients with NS, 3% overt hypothyroidism in patients with Nephrotic syndrome. Apparently, in patients with proteinuria, these urinary losses of thyroid hormones result in a stimulation of TSH production. In the study carried by Hajizadeh et al., was shown hypothyroidism requiring treatment in Sixty one cases identified as hypothyroid patients and was treated with supplementary levothyroxine [25], another study carried by Gilles, et al. shows that TSH is elevated in patients with NS. However, the clinical relevance of this finding is limited since overt hypothyroidism was present in less than 1% of the patients [26]. In the present study, frequency of death in children with Nephrotic syndrome about 4.6% cases of death more due to CKD& septicemia it complication cases of death due to septicemia reported in more than one study Kumar M, et al. and ISKDC [27].

The present study showed that incidence of side effects in the children receiving the steroid therapy were 56%. The most commonly side effectswas moon face found in (34.5%), follow by Gastritis (30%), short stature 22.4%, obesity 19.6%, Hirsutism 12%, cataracts 6.5%, and osteoporosis (2.8%). Lower than the study carried by Batishcheva GA et al. were recorded the incidence of side effects in the children receiving the steroid therapy 94.4%, while other study carried by Ishikura et al. hadthe following side effects short stature 13%, osteoporosis 13%, obesity 8%, cataracts 6%, and 6% hypertension [28]. This variation in the incidence of side effects of steroid therapy innephrotic patients may be due to a cumulative dose of prednisolone used, duration of steroid administration and regime of steroid tapering used forthose patients and other comorbid illness associated with N.S. In our studywe found that nephrotic patients associated with (hypothyroidism, sicklecell disease and diabetic mellitus) had increased incidence of short statureand obesity in SSNS compared with other studies [29]. Supraphysiologic doses of Glucocorticoids inhibit growth by multiplemechanisms including impaired release of growth hormone and decreased activity of insulin-like growth factor-1 (IGF-1) in growing bone. Whenglucocorticoid therapy can be reduced to lower; alternate-day dosing, catch-up growth is possible in these children [30-31]. In presented study, we also found that the complications with cyclophosphamide 27% both with oral and intravenous cyclophosphamide. Out of 18 patients, 5(27%) developed nausea and vomiting, and 4(22%) developed alopecia and 2(11%) developed leukopenia and none developedhemorrhagic cystitis.

A study conducted by Balaji and Ayyappan also found that the complications with cyclophosphamide both with oral and intravenouscyclophosphamide are less. Out of 27 patients, 4(14.8%) developed nausea and vomiting, and only 1(3.7%) developed alopecia and nonedeveloped leukopenia and hemorrhagic cystitis [31]. In the present study, it was found that the complications with Calcineurin inhibitor drug 35.7%. 9.5% developed elevated renal indices and 2.3% Tremor, 4.7% gingival hyperplasia, 14(33%) hirsutism and 2.3% developed hyperglycemia. Discontinuation of the drug (or reducedrug dose) was followed by normalization of serum creatinine in allchildren. In our study cyclosporine trough level was not done due to no available in our hospital, while other study carried by Mahmoud et al. reported hirsutism and gingival hyperplasia were the most frequent side-effects of Calcineurin inhibitor drug, occurring in 24(22.6%) and 55(51.9%) patients, respectively. While renal dysfunction identifiedin seven (6.6%) of all children and Mahmoud et al. study showdiscontinuation of the drug was followed by normalization of serumcreatinine in three children and the remaining child progressed to chronic renal failure [32], this can be explained by the difference in studied group, Mahmoud et al. study only patients with idiopathic FSGS while in ourstudy included primary and secondary NS. Study carried by Hazza I et al reported hirsutism and gingival hyperplasia were the commonest side effects encountered (in 8 and 7 of the 10 patients, respectively) , but no nephrotoxicity has been confirmed, this difference from current study can be explained Hazza I et al taken small sample size study (10 patients) and only SDNS include in study while our study include SRNS, SDNS and SSNS [33].

In current study the patients used mycophenolate drugs had adverse effects documented in 15.5 % patients, of whom the most frequently encountered were 15.5 % GIT problem (nausea, vomiting and or diarrhea) and 7.7% leucopenia. this is in agreement with other studycarried out by S Karunamoorthy et al., the majority of patients 88% had no adverse effects. Adverse events were documented the most frequentlydiarrhea and leucopenia[34] Study carried by K Afzal et al. noted the Prolonged use of MMF in patients with frequently relapsing or steroid-dependent NS is consideredsafe and associated with few adverse effects. The Side effects were limited to mild abdominal pain that resolved spontaneously. The absence of diarrhea, and hematological adverse effects in K Afzal study might be attributed to a lower dosage of the medication while in our study1200mg/SA/day [35]. In the present study, two patients used Rituximab, one patient of themdeveloped Genital herpetic lesion. While study carried by C. Trivin et al., among 98 patients treated with Rituximab, reported an infection rate of 21.5%. This difference can

be explained by long duration of study done by Trivin et al (eleven years) [36]. We did not record any side-effects or complications of albumin infusion,possibly because of the slow infusion of albumin and the administration ofdiuretics.

CONCLUSIONS

From this study it can be concluded that: Nephrotic syndrome is a chronic disease with a lot of complications like infections, renal impairment (AKI & CKD), Thromboembolism, hypertension complications and drug side effect. Frequency of relapse and duration of disease with response to steroid therapy are important predictors of increase risk of infections in Nephrotic patients.

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ORIGINAL ARTICLE

STATE OF AUTONOMIC REGULATION AND CEREBROVASCULAR REACTIVITY IN PATIENTS WITH HEADACHE WITH ARTERIAL HYPERTENSION

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ABSTRACT

The aim: A comprehensive assessment of the state of the autonomic nervous system and cerebrovascular reactivity of the cerebral blood flow in the patients with the headache accompanied by arterial hypertension (AH) and somatoform autonomic dysfunction (SAD).

Materials and methods: We conducted the clinical, autonomic regulation and Doppler sonography examination of 124 young patients (18-45 years old), including 51 men and 73 women in the conditions of the clinical base of the Kharkiv Medical Academy of Postgraduate Education in 2018-2021. All patients with cephalgias were divided into three groups: with AH stage II (Group I - 41 patients), AH stage I (Group II - 40 patients), SAD (Group III - 43 patients). The control group consisted of 50 patients of the corresponding gender and age

Results: The intensity of cephalalgia in patients with SAD was maximum. The autonomic tone (AT) was changed in 68.5%examined patients. It had a pronounced shift towards sympathicotonia. According to the visual analogue scale the maximum intensity of cephalgias was against the sympathicotonia. In the groups with organic lesions of the cerebral vessels the latent period delay was registered with the progression of the organic pathology. The regularity was revealed - the shortening of the evoked skin sympathetic potentials latency with the severity of cephalalgia, which can be interpreted as an increase in ergotropic effects with the realisation of the pain syndrome. The obtained data on the state of the AT indicate the depletion of the ergotropic processes with the progression of cerebral ischemia with a known increase in parasympathicotonia. In the patients of SAD group the CrCO2 and KrFNTvalues were significantly increased, in AH stage I group they slightly exceeded the standard values , in AH stage II group they were reduced. The reactivity to the orthostatic loads and functional metabolic test in all groups exceeded the control values.

Conclusions: 1. The cephalgic syndrome is one of the main symptoms of the autonomic dysfunction and arterial hypertension; the frequency and intensity of the headache increases with the hyperreactivity of the sympathetic system. 2. The SSP data indicate that the sympathetic activity triggers and maintains the pain syndrome, and can also be realized in the form of arterial hypertension. 3. The dysfunction of the central link of the GSR indicates the instability of the autonomous regulation, the work of the limbic-reticular complex, which is clinically manifested by the changes in the cerebral vascular tone. 4. The cerebrovascular hyperreactivity as a sign of the search for the optimal sanogenetic variant of the cerebral hemodynamics in patients with SAD and AH stage I occurs predominantly due to the vasodilatory component. 5. In the patients with AH stage II the vasoconstrictor reactions are observed with the depletion of the vasodilation reserves, which is a marker of the autoregulation failure.

KEY WORDS: Autonomic nervous system, headache, cerebrovascular reactivity, arterial hypertension, somatoform autonomic dysfunction

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INTRODUCTION

Arterial hypertension (AH) remains one of the predominant causes of the development of acute and chronic forms of the cerebrovascular pathology (CVP), the mortality, the main cause of disability, and deterioration in the quality of the life [1-3]. The disintegration of all levels of the autonomic nervous system (ANS) is one of the pathogenetic links of AH [4,5]. The balance of sympathetic-parasympathetic relationships is disturbed [6], it is clinically manifested by somatoform autonomic dysfunction (SAD), the latter is of a generalized systemic nature with the impaired cerebral blood flow due to inadequate adaptive capabilities against the increased blood pressure. At the same time the hyperactivation of the sympathetic nervous system is of particular importance, which contributes to cardiac and vascular changes with the development of complications of hypertension [7], which clinically leads to an increase in heart rate, an increase in vascular output during the transition to orthostasis, a change in baroreception, and vascular hypersensitivity to various stimulants [8]. The imbalance of autonomic control leads to a rapid depletion of the compensatory capabilities of the vascular system and the narrowing of its homeostatic range with the probable development of the so-called "diseases of adaptation" including cephalgia.

Headache (HA) is considered to be one of the leading symptoms of AH. In the European recommendations on

the diagnosis and treatment of AH special questionnaires for the HA detection during the patients` examination with hypertension are used [9]. The complaints on HA are presented in 44 to 87% of the patients with AH [10], mainly in the presence of the coexisting primary HA, most often, chronic tension headache and migraine [11].

Taking into consideration that the autonomic disorders are observed in the clinical picture of SAD and AH, it is necessary to study autonomic regulations for all levels of the response, state of tone and reactivity of autonomous system, including GSR for the clarification of the degree of adaptation, tactics of therapy and prognosis.

Cerebrovascular reactivity (CVR) is an integral indicator of the adaptive capacities of the cerebral circulation. The Doppler CVR study is informative in the patients with migraine and other types of headaches [12,13]. A combined study of the indicators of the autonomic regulation and CVR may be promising in the patients with the headache associated with an increase in AH.

THE AIM

The aim of the study was a comprehensive assessment of the state of the autonomic nervous system and cerebrovascular reactivity of the cerebral blood flow in the patients with the headache accompanied by hypertension and somatoform autonomic dysfunction.

MATERIALS AND METHODS

124 patients aged 18 to 45 years (56 men, 68 women) with hypertension with the persistent or periodic increase in blood pressure in the conditions of the clinical base of the Kharkiv Medical Academy of Postgraduate Education in 2018-2021 were examined. All patients with cephalgias were divided into three groups: with AH stage II (Group I - 41 patients), AH stage I (Group II - 40 patients), SAD (Group III - 43 patients). The questionnaire was used to identify the signs of the autonomic changes in order to identify the autonomic dysfunctions. The study of the autonomic tone (AT) was performed using the table "24 stigmas" for the rapid diagnosis of AT. The intensity of cephalalgias was studied using the visual analogue scale (VAS), and their characteristics were studied using the questionnaire form for the HA evaluation. The evoked skin sympathetic potentials (SSP) were registered along with the study of the amplitude of the second phase (A_2), as well as the duration of the latent period (LP).

The study of the cerebrovascular reactivity was carried out on the ultrasound scanner Ultima-PA (RADMIR, Ukraine), using the following functional loads and reactivity coefficients: hypercapnic (KrCO2), hyperventilation (KrO2), orthostatic (KrOL), anti-orthostatic (KrAOL), functional nitroglycerin test (KrFNT), functional metabolic test (KrFMT) .

The control group (CG) included 50 clinically healthy male and female volunteers of the corresponding age.

The program Statistica 8.0 was used for the statistical processing with the Wilcoxon as well as the Mann-Whitney U criterion assessment. The differences at $p < 0.05$ were considered statistically significant. The study complies with the requirements of the Helsinki Declaration and is approved by the ethics commission of the Kharkiv Medical Academy of Graduate Education.

RESULTS

All patients underwent a neurological examination with the diagnosis of the type of the headache in accordance with the ICHD-3 (2018). According to the structure HA were distributed between the groups of the patients as follows. Group I: headache associated with arterial hypertension - 22 patients (53.7%), tension headache - 19 patients (46.3%), Group II: tension headache - 16 patients (40.0%), migraine - 13 patients (32.5%), headache associated with arterial hypertension - 11 patients (27.5%); Group III: tension headache - 18 patients (41.9%), mixed headache (tension headache involving pericranial muscle and cervicogenic headache) - 14 patients (32.5%), migraine - 11 patients (25.6%).

In all groups cephalgias were maintained or increased in the presence of the degenerative-dystrophic changes in the cervical spine with muscle tone reactions in the occipital region.

The results obtained in the relation to the intensity of the pain in the studied groups indicate that the average VAS scores were slightly higher in Group II (by 0.27 points) compared to Group I whenever they were present. At the same time in Group III the intensity of cephalalgia was maximum and its average values reached 4.5±0.8 points, and it was more than 1.2 times higher than in Group I ($P< 0.05$).

AT was changed in 85 (68.5%) examined patients, in Group I - 27 (68.4%), in Group II - 31 (77.5%), in Group III - 27 (62.8%). It had a pronounced shift towards sympathicotonia: in Group III SAD - 21 (48.8%) with a minimum in vagotonia (3 - 7.0%), in groups with AH, a similar distribution remained in Group II - 21 (51.2%) versus 6 - (14.6%), however, Group I demonstrated a significant inversion ($P<0.05$) of these indicators with the "leveling" of the number of pathological values of AT: 16 (40.0%) versus 16 (40.0%), which indicates some weakening of ergotropic and an increase in trophotropic effects with a sharp decrease ($P <0.05$) in the normal distribution of AT in the process of the progression of the organic pathology of the brain (Fig. 1).

According to the VAS, the maximum intensity of cephalgias was against the sympathicotonia: 4.2±0.09 points (Group II) and 4.3±0.11 points (Group I), $P<0.05$; 4.5±0.08 points (Group III), $P< 0.05$. (Fig.2).

In the study of the galvanic skin reflex (GSR) according to the registration of SSP in Group III with SAD the average values of the LP duration were 1.29 ± 0.07 s and were minimal. In the groups with organic lesions of the cerebral vessels the LP delay was registered with the progression of the organic pathology: from 1.41±0.07 (Group II) to 1.75±0.09 s (Group I), $P<0.05$.

The regularity was revealed - the shortening of the SSP latency with the severity of cephalalgia, which can be interpreted as an increase in ergotropic effects with the realisation

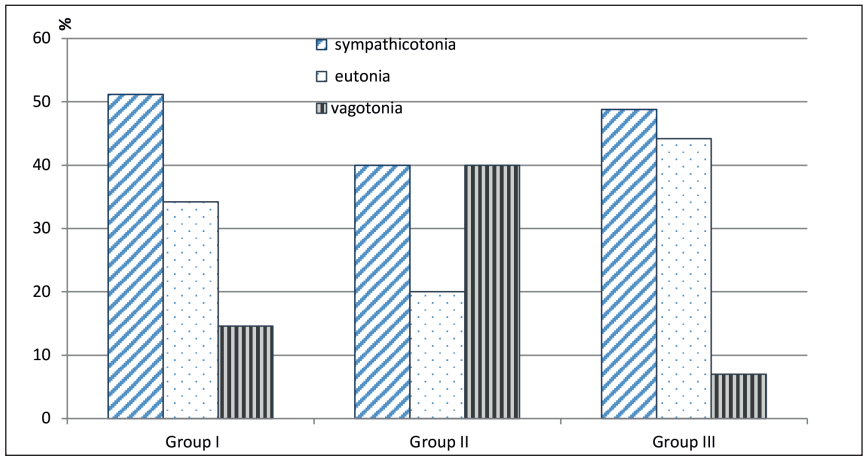

Fig. 1. The relative distribution of AT in the studied groups

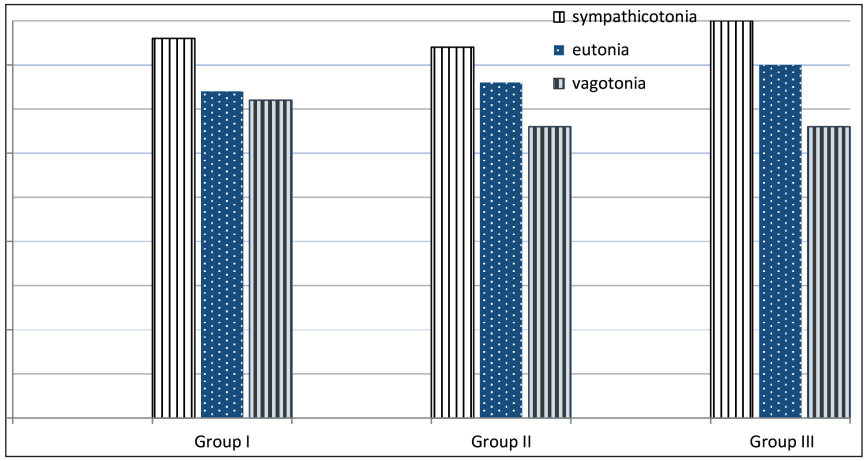

Fig. 2. The intensity of headache in groups depending on the initial autonomic tone (VAS points).

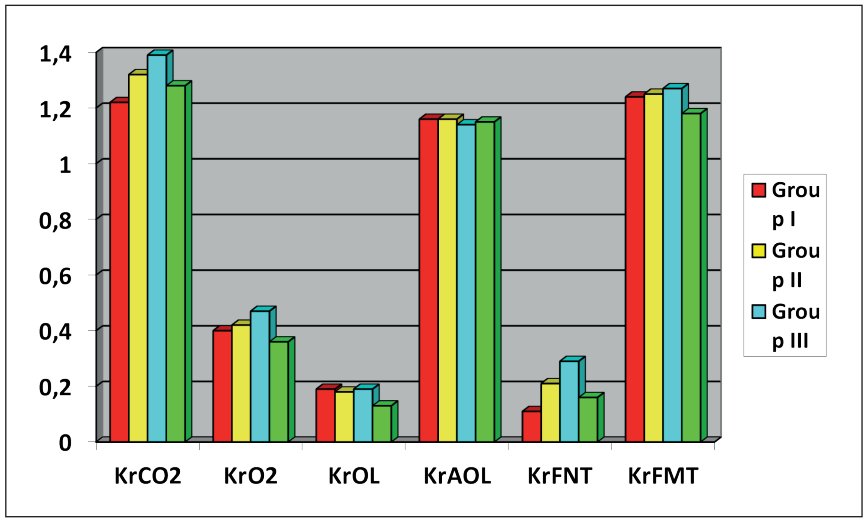

Fig. 3. CVR values in patients with the headache

of the pain syndrome. At the same time, with a low-intensity cephalalgia, the LP lengthened, which is apparently associated with trophotropic inhibition of the development of cephalgia (Table I).

The obtained data on the state of AT indicate the depletion of the ergotropic processes with the progression of cerebral ischemia with a known increase in parasympathicotonia (Table II).

A clear pattern of the decrease in the amplitude of the second phase of GSR - A_2, depending on the depletion of the ergotropic system was revealed (Table III). The average values of A_2 in Groups II and I were below standard values (2.66 ± 0.1 and 2.55 ± 0.2 mV, $p < 0.05$), however, in Group III they were close to the standard and equal to 2.71±0.2 mV.

The significant difference between A_2 indicators by 2 or more times in all examined groups ($p < 0.05$) indicates that, regardless of the etiopathogenesis of vascular lesions, ANS impacts and/or accompanies the pathological process, indicating the state of the body's adaptive capabilities, which may predetermine the prognosis and prospects for therapy.

In the patients of Group III the CrCO2 values were significantly increased compared to the CG (1.39±0.05, CG - 1.28±0.04; $p < 0.05$), in Group II they slightly exceeded the

Table I. The distribution of LP indicators in groups depending on the intensity of cephalgias

Cephalgia (VAS)	SSP latency (sec)		
	Group I	Group II	Group III
High intensivity (4 + points)	1,31±0,06	1,51±0,09	1,21±0,06
Low intensivity (1-3 points)	1,75±0,4*	2,20±0,12*	1,60±0,20*

*p <0,05 compared to average data

Table II. The change in SSP LP depending on the initial AT in groups

Groups	SSP latency (sec)			
	Total	Sympathicotonia	Eutonia	Vagotonia
Group I (n=41)	1,41±0,10	1,24±0,12	1,52±0,14	1,88±0,13*
Group II (n=40)	1,78±0,11	1,52±0,11	1,74±0,12	2,11±0,14*
Group III (n=43)	1,31±0,12	1,17±0,09	1,44±0,05	1,80±0,09*

*p <0,05 compared to average data

Table III. The change in SSP A2 depending on the initial AT in groups

Groups	Amplitude (A_2) SSP (mV)			
	Total	Sympathicotonia	Eutonia	Vagotonia
Group I	2,69±0,2*	3,43±0,3	2,11±0,4	1,55±0,2**
Group II	2,56±0,3*	3,65±0,1	2,17±0,5	1,81±0,3**
Group III	2,73±0,1	3,58±0,2	1,88±0,5	1,61±0,1**

*p <0,05 compared to standart average data
**p <0,05 compared to maximum-minimum

standard values (1.32±0.03), in Group I they were reduced (1.22±0.05). The parameters of CrFNT changed in groups in a similar way: a significant increase in Group III (0.29±0.05, CG - 0.16±0.04; p <0.05), a slight increase in Group II (0.21±0.04) and a decrease in Group III (0.11±0.03, p<0.05), the KrO2 indicators also exceeded the standard ones, but to a lesser extent than the KrCO2 data (0,46±0.07, CG - 0.36±0.03). The reactivity to the orthostatic loads and functional metabolic test in all groups exceeded the control values. The response to the antiorthostatic test was within the standard limits (Fig. 3).

DISCUSSION

It has been found out that HA is a key manifestation of autonomic dysfunctions and arterial hypertension. The HA intensity slightly decreased as the pathological process progressed in the AH groups, while at the same time it was maximum in somatoform dysfunctions. The pathological autonomic tone prevailed in all groups. The frequency and intensity of cephalalgia increased with the presence of sympathicotonia, especially in the SAD and AH stage I groups. At the same time with the growth of structural damage to the CNS (AH stage II) trophotropic (vagal) manifestations intensified, and cephalalgias in all groups were less intense, which can be regarded as a decrease in the adaptive capabilities of the body as a whole.

From the data obtained regarding the shortening of latency during intense headaches, one can assume an increase in ergotropic effects, i.e. sympathetic activity apparently triggers and maintains the pain syndrome, and can also be realized in the form of arterial hypertension.

At the same time, with low-intensity cephalalgia the LP has lengthened, which is apparently associated with trophotropic effects. Thus, the dysfunction of the ANS has a leading influence on the development of HA of various origins.

The involvement of suprasegmental autonomous structures has been registered, i.e. the central link of the GSR with the corresponding central delay in the form of a "spring stretching phenomenon" can be interpreted as instability of autonomous regulation, the work of the limbic-reticular complex, which is clinically manifested by a change in the vascular tone of the brain.

The autoregulatory reserve in case of AH episodic increase (in SAD patients) and AH stage I is characterized by pronounced hyperreactivity in almost all components. In patients with AH stage II there is a decrease in the response to CO2 and FNT, which is associated with the depletion of vasodilation reserves. The changes in the reactivity along the myogenic circuit with AH progression are similar to the data of the metabolic circuit of autoregulation. An increased response to O2-test, orthostatic test and FMT in all groups indicates predominantly vasoconstrictor reactions in patients with HA. It can be assumed that the exhaustion of vasoconstriction reserves in these patients occurs later than similar changes in the vasodilator component.

Our data are also confirmed by the studies of other authors indicating an increase in the sympathetic activity in cephalgias and potentiation of the development of AH [7,8]. In addition, the obtained results correspond to the

hypothesis that the autonomic dysfunctions are an additional factor in the development of some cephalalgias, primarily migraine paroxysms [14]. The identification of this kind of imbalance of tropho- and ergotropic influences can be effectively performed with the help of SSP.

CONCLUSIONS

1. The cephalgic syndrome is one of the main symptoms of the autonomic dysfunction and arterial hypertension; the frequency and intensity of the headache increases with the hyperreactivity of the sympathetic system.
2. The SSP data indicate that the sympathetic activity triggers and maintains the pain syndrome, and can also be realized in the form of arterial hypertension.
3. The dysfunction of the central link of the GSR indicates the instability of the autonomous regulation, the work of the limbic-reticular complex, which is clinically manifested by the changes in the cerebral vascular tone.
4. The cerebrovascular hyperreactivity as a sign of the search for the optimal sanogenetic variant of the cerebral hemodynamics in patients with SAD and AH stage I occurs predominantly due to the vasodilatory component.
5. In the patients with AH stage II the vasoconstrictor reactions are observed with the depletion of the vasodilation reserves, which is a marker of the autoregulation failure.

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ORIGINAL ARTICLE

PAIN SYNDROME IN PATIENTS AFTER COMBINED OPERATIONS FOR COMBINED ANORECTAL DISEASES USING MODERN SURGICAL TECHNOLOGIES

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ABSTRACT

The aim: To compare the pain in patients with combined pathology of the anal canal and rectum after combined operations using modern high-frequency electrosurgical and radio-surgical devices and a conventional metal scalpel.

Materials and methods: The results of treatment of 635 patients with combined pathology of the anal canal and rectum using high-frequency electrosurgery and radio-wave surgery, which were divided into 4 study groups, as well as 112 patients using a metal surgical scalpel were analyzed.

Results: Assessing the pain syndrome on the first day of the postoperative period, it was found that it was most pronounced in control group patients, where the need for analgesia 2 % promedol solution was 4±1 ml and in the first, third and fourth study groups patients needed for analgesia 2±1 ml of 2 % promedol solution when using "Surgitron" radio-wave surgery device, "EFA" and "KLS Martin" high-frequency electrosurgical devices respectively. When using device "ERBE ICC 200", the need for a 2 % promedol solution for analgesia was 3±1 ml.

Conclusions: Using of radio-wave surgery device and high-frequency electrosurgery devices is much better compared to the use of a surgical metal scalpel because they cause much less pain due to the formation of a thin layer of coagulation necrosis, promoting the formation of a delicate elastic scar and preventing formation of scar anal strictures.

KEY WORDS: pain syndrome, combined anorectal pathology, anal canal, rectum

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INTRODUCTION

Over the last two decades, there has been a tendency to significantly increase the combined pathology of the anorectal area, which requires surgical treatment. Thus, in 18–32 % of patients admitted to proctology hospitals, there is a combination of two - three diseases of the anorectal area, which are subject to surgical treatment [1]. The most common combination of hemorrhoids with anal fissure – 12.3–59.1 %, hemorrhoids with anal fistula – 29.3–40.1 %, hemorrhoids with anal polyp – 13.2–18.4 % [2].

The main method of surgical treatment of the most common proctological diseases - hemorrhoids, anal fissures and fistulas is traditional instrumental surgery, but it is often accompanied by severe postoperative pain, which according to some authors, is directly related to surgical trauma in the innervation zone of the anal canal, causing local edema, acute subclinical infection and inflammation [3]. Other authors claim that postoperative pain occurs due to compression of the smooth muscle fibers of the internal sphincter and mucous membrane in the area of stitching with the development of subsequent tissue reaction to the suture, which is most common after closed hemorrhoidectomy [4, 5].

Due to the progressive development of modern technologies in coloproctology, new methods of surgical treatment of pathology of the anal canal and rectum began to be introduced. One of the most common methods of tissue exposure in colorectal surgery was monopolar electrocoagulation, which allowed coagulation of vessels up to 1 mm in diameter [6]. It provided good hemostasis, but, at the same time, caused, especially with open hemorrhoidectomy, intense postoperative pain, edema of perianal tissues, anal stenosis. All the above complications occurred due to deep thermal tissue damage, which sometimes reached even 9 mm [7].

The "LigaSure" electrothermal systems of Valleylab (USA) and "En Seal" of Ethicon (USA) for coagulation and cross-section of vessels up to 7 mm in diameter have become quite widely used in coloproctological practice. They provided thorough hemostasis, reduced the duration of surgery and eliminated the need for isolation and ligation of the vascular leg of the hemorrhoid, as well as reduced the time of rehabilitation of patients [8, 9]. But, at the same time, these techniques had a number of disadvantages: postoperative bleeding (1.6–2.5 %), anal stricture (2.8 %), recurrence of diseases (7.5 %), as well as severe pain (2.1–11.7 %) [10–12].

Using of ultrasonic scalpel Harmonic Focus by Johnson & Johnson (USA) for the treatment of anorectal pathology

made it possible to reduce the depth of coagulation tissue necrosis to 1.5 mm, but, unfortunately, did not avoid postoperative pain, which occurred with a frequency of 5-6.6 % [13]. In addition, this method was accompanied by postoperative bleeding (6.1 %) and recurrence of diseases (9.1 %) [14, 15].

Since 2000, laser technology has been widely used in the treatment of anal fissures and chronic hemorrhoids. Using of laser irradiation with the infrared part of the spectrum reduces the duration of operations, promotes minimal bleeding of dissected tissues, reduces the intensity of pain, as well as the number of postoperative complications [16]. At the same time, studies on the use of lasers for hemorrhoidectomy have shown that this method is accompanied by the formation of deep postoperative wounds with a fairly long healing, which is probably due to the uncontrolled depth of thermal exposure, which can sometimes reach 4.2 mm, causing quite intense pain syndrome [17]. It is also known that carbon dioxide lasers do not have sufficient hemostatic properties in the presence of blood in the surgical wound and carry out direct penetration of infrared energy into the tissue, causing it to overheat [18].

Thus, the urgency of the problem of combined pathology of the anal canal and rectum is quite high and contributes to the development and implementation in clinical practice of new modern and effective methods of surgical treatment of this pathology, which would have minimal impact on tissues, preventing severe pain and various postoperative complications in postoperative period and would contribute to the rapid medical and social rehabilitation of patients.

THE AIM

The aim of the study was to conduct a comparative assessment of pain in patients with combined pathology of the anal canal and rectum after combined operations using modern high-frequency electrosurgical and radiosurgical devices, as well as a conventional metal scalpel.

MATERIALS AND METHODS

In the period from January 2007 to March 2020 in the Department of Proctology of Public Non-Profit Enterprise "Khmelnytskyi regional hospital" under Khmelnytskyi Regional Council were operated on 635 patients with combined pathology of the anal canal and rectum using the device of radio-wave surgery "Surgitron", as well as high-frequency electrosurgical devices "ERBE ICC 200", "EFA" and "KLS Martin". Of these, 358 (56.4 %) patients were male and 277 patients (43.6 %) were female. The age of patients ranged from 18 to 76 years.

All 635 patients, who were divided into 4 study groups, signed a voluntary informed consent for anesthesia and surgery, which were performed under spinal anesthesia.

The first study group consisted of 245 patients with combined pathology of the anal canal and rectum, who were operated using a radio-wave surgery device "Surgitron" for the period from September 2009 to February 2019. Of these, 143 (58.4 %) patients were male and 102 (41.6 %) were female. The age of patients ranged from 18 to 74 years.

At the heart of the radio-surgery device "Surgitron F.F.P.F. EMC "manufactured by the American company Ellman International is the effect of converting electric current on a radio wave with an output frequency of 3.8-4.0 MHz, under the influence of which the cut tissue resists the penetration of radio waves, while emitting heat, under the influence of which tissue cells are in the path of waves, disintegrate and evaporate and the fabric seems to "disperse". This feature of the device of radio-wave surgery with the above-stated frequency of waves causes soft influence on fabrics with their minimum damage.

The second study group consisted of 169 patients with combined pathology of the anal canal and rectum, who were operated using a high-frequency electro-surgery device "ERBE ICC 200" for the period from March 2008 to February 2019. Of these, 104 (61.5 %) patients were male and 65 (38.5 %) were female. The age of patients ranged from 20 to 76 years.

At the heart of the high-frequency electro-surgery device "ERBE ICC 200" with an output frequency of 330 KHz and a nominal power of 50 - 80 W at 200 - 500 Ohms, there is an automatic power control system that recognizes low-impedance loads, regulating the operation of high-frequency generator the required intensity of high-frequency voltage.

The third study group consisted of 114 patients with combined pathology of the anal canal and rectum, who were operated using high-frequency electrosurgery "EFA" for the period from January 2007 to February 2019. Of these, 65 (57 %) patients were male and 49 (43 %) were female. The age of patients ranged from 24 to 72 years.

The high-frequency electro-surgery device "EFA" has a system of adaptive control of the output high-frequency voltage depending on the resistance of the tissue with stabilization of the output power, which is 200W at a frequency of 375kHz in a wide range of loads (from 100 to 2000Ohm). This unique feature of the device allows to carry out an electrotomy and electrocoagulation with the maximum effect and the minimum necrosis of tissues and also allows to use it in liquid environments.

The fourth study group consisted of 107 patients with combined pathology of the anal canal and rectum, who were operated using a high-frequency electro surgery device "KLS Martin" for the period from October 2017 to February 2020. Of these, 43 (40.2 %) patients were male and 64 patients (59.8 %) were female. The age of patients ranged from 19 to 65 years.

An important feature of the "KLS Martin" high-frequency electro-surgery device with an output frequency of 450 kHz is the presence of a mixed cutting mode with marginal coagulation effect, as well as a "spray-coagulation" mode, which provides fast and uniform hemostasis with minimal carbonization, which improves healing deep necrosis.

The control group consisted of 112 patients with combined pathology of the anal canal and rectum, who were operated traditionally with a surgical metal scalpel.

Evaluation of pain intensity on the first day of the postoperative period was performed in 30 patients from each study and control groups by calculating their needs for narcotic analgesics (2 % promedol solution). Also, subjective assessment of pain was performed by patients themselves on days 1, 3, 5 and 7 using a visual analog scale (VAS), in which pain intensity was assessed from 0 (complete absence of pain) to 10 (excruciating pain).

After surgery using radio-wave surgery device "Surgitron" and high-frequency electro-surgery devices "ERBE ICC 200", "EFA" and "KLS Martin", 30 patients from each study group underwent morphological examination of anal canal and rectal tissues for examination. Measurement of the thickness of the coagulation necrosis layer was performed using an eyepiece-micrometer scale.

The operating material was fixed in 10 % neutral formalin solution. Next, the material was produced in a carousel histoprocessor type STP-120, for filling paraffin blocks used EC-350 station, for cutting paraffin blocks – rotary microtome series HM - 340E, for staining histological specimens – Robot-Stainer HMS-740 (all devices from Carl Zeiss MICROM International GmbH). The drugs were stained with hematoxylin and eosin. An Axioskop 40 microscope with an Axio Cam MRc5 camera (Karl Zeiss) was used.

Statistical analysis of the obtained data was performed using IBM SPSS STATISTICS SUBSCRIPTIONAL TRIAL software. License number: L-CZAA-BHG85V. The connection between the depth of coagulation necrosis and the severity of pain for each group, as well as for the general group, which included patients of all study groups was studied. Because the distribution of pain in the depth of the coagulation necrosis layer differed from normal, Spearman's correlation coefficient was used to assess the connection. To confirm also the relationship between the depth of coagulation necrosis and the severity of pain, all groups were divided into subgroups in which the severity of pain was the same. Comparison of subgroups was performed using the Kraskel-Wallis test. The critical level of statistical significance was 0.05.

RESULTS

As a result of the study it was found that in 473 patients (74.6 %) were diagnosed 2 pathologies of the anal canal and rectum, in 146 (22.9 %) - 3 pathologies, in 15 (2.4 %) - 4 pathologies and in 1 (0.1 %) – 5 pathologies of the anal canal and rectum.

The study identified 57 variants of combined pathology of the anal canal and rectum, among which in the control and in all 4 study groups the most common were the following 10: chronic anal fissure and anal polyp – 110 (17.3 %); chronic anal fissure and combined hemorrhoids – 80 (12.6 %); combined hemorrhoids and anal fistula – 62 (9.7 %); combined hemorrhoids and anal polyp – 61 (9.6 %); chronic anal fissure, anal polyp and hypertrophied perianal skin tags – 38 (5.9 %); combined hemorrhoids, chronic anal fissure and anal polyp – 32 (5.0 %); external hemorrhoids and anal polyp – 22 (3.5 %); external hemorrhoids and chronic anal fissure – 18 (2.8 %); anal polyp and hypertrophied perianal skin tags – 14 (2.2 %); combined hemorrhoids, chronic anal fissure and anal polyp – 10 (1.6 %).

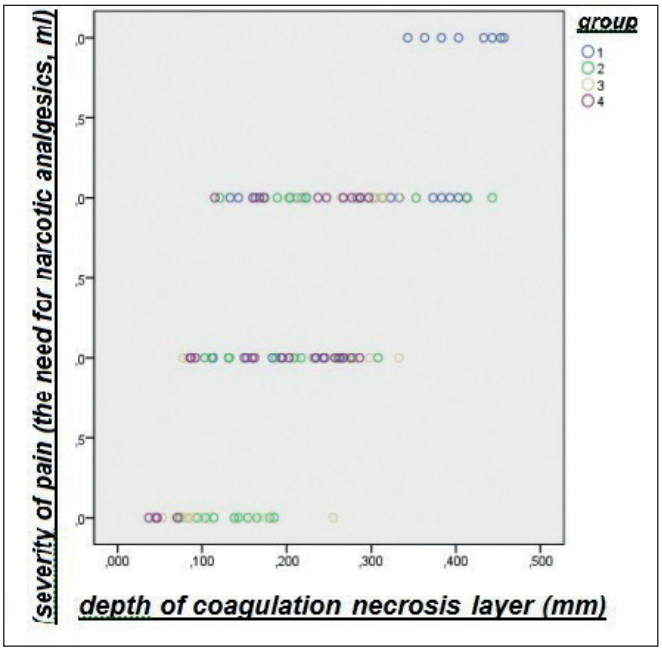

Fig.1. Skaterogram for groups 1 – 4

The nature of the performed surgical interventions depended on the variant of combined pathology of the anal canal and rectum. Patients underwent the following combinations of surgical interventions: excision of the anal fissure and polypectomy – 110 (17.3 %); hemorrhoidectomy and excision of the anal fissure – 80 (12.6 %); hemorrhoidectomy and excision of the anal fistula – 62 (9.7 %); hemorrhoidectomy and polypectomy – 61 (9.6 %); excision of the anal fissure, polypectomy and electroexcision of hypertrophied perianal skin tag – 38 (5.9 %); hemorrhoidectomy, anal fissure excision and polypectomy – 32 (5.0 %); hemorrhoidectomy and polypectomy – 22 (3.5 %); hemorrhoidectomy and excision of the anal fissure – 18 (2.8 %); polypectomy and electroexcision of hypertrophied perianal skin tag – 14 (2.2 %); hemorrhoidectomy, anal fissure excision and polypectomy – 10 (1.6 %) patients.

In the postoperative period, patients in the study group showed no signs of scar stricture of the anal canal, while in the control group in 2 (1,8 %) patients showed the formation of scar stricture of the anal canal, which required surgical measures to eliminate them.

When assessing the pain syndrome on the first day of the postoperative period, it was found that it was most pronounced in patients of the control group, where the need of 2 % promedol solution for analgesia was 4±1 ml and in the first, third and fourth study groups patients needed 2±1 ml of 2 % promedol solution when using the radio surgery device "Surgitron" and high-frequency electrosurgical devices "EFA" and "KLS Martin" respectively. When using high-frequency electro surgery device "ERBE ICC 200", the need for a 2 % solution of promedol for analgesia was 3±1 ml.

Table I. Comparative characteristics of the severity of pain in patients of the control and study groups

The day after the operation	The severity of pain in points				
	Control group (metal scalpel)	Study group №1 (Surgitron)	Study group №2 (ERBE ICC 200)	Study group №3 (EFA)	Study group №4 (KLS Martin)
1	4,1±1,5	1,7±0,9	2,8±1,1	2,4±0,9	1,9±1,0
3	3,6±1,2	1,5±0,7	2,5±1,2	2,2±0,7	1,7±0,6
5	3,1±1,1	1,4±0,6	2,1±0,6	1,9±0,5	1,5±0,7
7	2,8±1,0	1,2±0,6	1,9±0,8	1,5±0,4	1,3±0,6

Table II. Evaluation of the relationship between the severity of pain and the depth of the layer of coagulation necrosis

Group	Sample size	Spearman's correlation coefficient (ρ)	Significance	Direction of communication	The power of communication
1	30	0,56	0,001	Direct	Average
2	30	0,71	<0,001	Direct	Average
3	30	0,58	0,001	Direct	Average
4	30	0,73	<0,001	Direct	Average
General	120	0,69	<0,001	Direct	Strong

Table III. Comparison of subgroups using Kraskel-Wallis test

Group	Meaning
1	0,003
2	0,001
3	0,008
4	<0,001
General	<0,001

The severity of pain in points in patients of the control and study groups at different times of the postoperative period (1,3,5,7 days) are presented in Table I.

The dependence of the pain syndrome intensity on the depth of the coagulation necrosis layer in the groups is presented in Table II and Table III.

For all groups, the connection between the severity of the pain syndrome and the depth of the coagulation necrosis layer is statistically significant and for all groups the correlation is direct (as one indicator increases, so does the other) (Fig.1).

Thus, for groups 1 and 3 the correlation is medium, for groups 2 and 4 - strong.

During the morphological study it was found that using of radio-wave surgery device «Surgitron» in patients of the first study group contributed to the preservation of tissue structure with the formation of the thinnest layer of coagulation necrosis among all study groups, the average depth of which was 0,189±0,085 mm. When using the high-frequency electrosurgical device «KLS Martin» in patients of the fourth study group tissue preservation is also observed with the formation of a thin layer of coagulation necrosis along the incision edge, which was slightly deeper than in the first group with a thickness of 0,194±0,090 mm. After the application of the high-frequency electrosurgery device «EFA» in patients of the third study group formed a deeper layer of coagulation necrosis than in the first and fourth groups, the average thickness of which was 0,208±0,097 mm. The deepest layer of coagulation necrosis among all groups was after the application of the high-frequency electrosurgery device «ERBE ICC 200», the average thickness of which was 0,302±0,107 mm.

Using of a surgical metal scalpel in patients of the control group was accompanied by mechanical trauma to the tissues with the formation of common hemorrhages.

DISCUSSION

Due to using of radio-wave surgery device "Surgitron" with an output frequency of 3,8–4,0 MHz for the treatment of patients with combined pathology of the anal canal and rectum, the thinnest layer of coagulation necrosis is formed in the tissues, the smallest depth of which was even 0,037 mm resulting patients of the first study group on the first day after surgery had the lowest pain, amounting to 1,7±0,9 points, which corresponded to the weakest pain on the VAS scale and they needed for analgesia only 2±1 ml of 2 % promedol solution.

Application of high-frequency electrosurgery device "KLS Martin" with an initial frequency of 450 kHz was also accompanied by the formation of a thin layer of coagulation tissue necrosis with a depth of 0,053 to 0,334 mm, so that patients pain in the fourth study group on the first day after surgery was weak on the VAS scale being 1,9±1,0 points and they also needed for analgesia only 2±1 ml of 2 % promedol solution.

The effect on the tissues of high-frequency electrosurgery device "EFA" with an initial frequency of 375 kHz was deeper than in the above groups, forming a layer of coagulation tissue necrosis with a depth of 0,074 to 0,434 mm, resulting in patients of the third study group pain after surgery amounting to 2,4±0,9 points but they required for analgesia also (2±1ml) 2 % promedol solution.

Using of high-frequency electrosurgical device "ERBE ICC 200" with an output frequency of 330 kHz was accompanied by the formation of the deepest layer of coagulation necrosis than in above groups, which ranged from 0,113 to 0,453 mm, causing patients pain in the second study group was on the borderline between weak and moderate on the VAS scale, amounting to 2,8±1,1 points and patients in this group needed 3±1 ml of 2 % promedol solution for analgesia.

The results indicate that using of the above modern radiosurgical and high-frequency electrosurgical technologies is accompanied by a much smaller depth of coagulation necrosis than the use of devices "Liga Sure", "En Seal" and Harmonic Focus, the depth of which on tissues according to some authors is from 1,5mm to 2mm [9, 11, 13].

Mechanical trauma to the tissues with the formation of common hemorrhages when using a surgical metal scalpel in patients of the control group was accompanied by the emergence of moderate pain on the VAS scale on the first day after surgery, which was 4,1±1,5 points and patients in the control group needed the most 2 % solution of promedol, which was 4±1 ml.

The decrease in the intensity of the pain syndrome was observed on the 3rd day after surgery in all study groups and in the control group in parallel, amounting to 1,5±0,7, 2,5±1,2, 2,2±0,7, 1,7±0,6 and 3,6±1,2 points respectively on the VAS scale. And on the 5th day of the postoperative period, the lower limit of pain intensity in the first and fourth groups was 0,8 points, which indicated its barely noticeable severity, while in the second and third groups it was 1,5 and 1,4 points respectively which corresponded to mild pain on the VAS scale. In the control group on the 5th day the pain syndrome was on the border between weak and moderate on the VAS scale, amounting to 3.1±1.1 points. Almost complete absence of pain on the 7th day after surgery was observed in patients of the first (0,6 points) and the fourth study group where the lower limit of pain was 0,6 points and 0,7 points respectively. In patients of the control group, second and third study groups the intensity of pain also decreased, being 2,8±1,0 points, 1,9±0,8 points and 1,5±0,4 points respectively which corresponded to mild pain on the VAS scale.

Thus, the severity of pain in the study groups and even in the control group was significantly lower than the pain in studies of other authors, which ranged from 5,8±1,6 to 8,7±1,4 points [3–5].

CONCLUSIONS

1. Using of radio-wave surgery device "Surgitron" with a wave frequency of 3,8–4,0 MHz as well as the "KLS Martin" high-frequency electrosurgery device with a frequency of 450 kHz during combined operations in patients with combined pathology of the anal canal and rectum is accompanied by the least damage of tissues contributing to the formation of a thin layer of coagulation necrosis which is from 0,037 to 0,334mm, causing the presence of mild pain on the 1st day after surgery and its almost complete absence on the 7th day of the postoperative period.
2. Application of high-frequency electrosurgical devices "EFA" with a frequency of 375 kHz and "ERBE ICC 200" with a frequency of 330 kHz during combined operations in patients with combined pathology of the anal canal and rectum is accompanied by a slightly greater damaging effect on the tissue from 0,074 to 0,453mm, causing the appearance of more intense pain (to the level of moderate on the VAS scale) on the 1st day after surgery and its gradual reduction to the level of weak on the 7th day of the postoperative period
3. Application of these modern devices of high-frequency electrosurgery and radio-wave surgery is much better compared to the use of a surgical metal scalpel because they cause much less pain due to the formation of a thin layer of coagulation necrosis, promoting the formation of a delicate elastic scar and preventing of scar stricture occurence of the anal canal in postoperative period.

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ORIGINAL ARTICLE

ASSOCIATION OF SCREENING MARKERS OF COAGULATION WITH THE SHORT-TERM OUTCOME IN THE SMALL BOWEL OBSTRUCTION IN ADULTS: A RETROSPECTIVE STUDY

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ABSTRACT

The aim: To evaluate the possibility of using screening markers of coagulation to the assessment of severity and predict short-term outcomes in patients with small bowel obstruction.

Materials and methods: The study was based on the results of treatment of 71 patients 18-60 years old in 2019-2021. Patients were divided into two groups: in the 1st included those with a positive outcome (90.1%), and in the 2nd those with adverse outcomes (9.9%).

Results: Only the laparoscopy approach has been in 12.5%, the laparotomy in 78.9%, and the hybrid in 9.9% of patients. There were no significant differences in screening tests of coagulation function indicators, including D-dimer, fibrinogen, Activated Partial Thromboplastin, International Normalised Ratio levels, and the International Society on Thrombosis and Hemostasis Criteria (ISTHC) score in two groups of patients before surgery. The predictive value of preoperative Sequential Organ Failure Assessment (SOFA) data (AUC = 0.844), serum lactate (AUC = 0.805), and systolic blood pressure (SPB) data (AUC = 0.808) before surgery were significant. The SOFA (AUC = 0.844) and APACHE II scores (AUC = 0.802), serum lactate (AUC = 0.884), D-dimer (AUC = 0.812), Antithrombin (AUC = 0.815), and CRP (AUC = 0.856) levels, SPB (0.856) within the first 72 hours after surgery were also good predictors of short-term outcomes (P = 0.000).

Conclusions: It was confirmed that three parameters were predictors of early mortality before surgery, none of them included parameters of coagulation and seven parameters via 72 hours after surgery, which had included some parameters of coagulation.

KEY WORDS: small bowel obstruction, parameters of coagulation, severity, prognosis, surgery, mortality

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INTRODUCTION

Surgical treatment small bowel obstruction (SBO) remains one of the most challenging problems of emergency abdominal surgery nowadays, most studies have shown that one is a heterogeneous syndrome caused by an imbalanced host response to obturation and ischemia/necrosis of the small intestine. In all of these cases, the pathological processes may lead to ischemia, necrosis, intestinal perforation, peritonitis, sepsis [1, 2]. It has been reported that SBO accounts for 12% to 16% of surgical hospitalizations, about 40% of cases are associated due with strangulation of the small intestine, at least 300,000 surgical procedures are performed annually in the United States [3]. Nonviable asphyxiation of the small intestine accounts for 16% of SBO, which four times increase the risk of death in comparison with the rates in patients with viable asphyxiation [4], and in patients with strangulation, SBO had a mortality rate of 2 to 10 times higher than patients with non-strangulation [5]. It was noted that the violation of macro-and microcirculation in the early stages before and after surgery in patients with SBO contributes to significant shifts in homeostasis and plays a major role in the development of multiple organ disorders due to frequent disseminated and often uncontrolled activation of coagulation. Lots of pathological conditions can also cause disseminated intravascular coagulation, with sepsis, organs destruction (e.g., severe pancreatitis, necrosis of the large intestine, reperfusion syndrome, cancer, trauma, etc.) that being among the most common triggering factors [6,7]. The prevalence of these disorders depends on the conditions of hospitalization, timing onset of diseases and is higher in critically ill patients admitted to intensive care units [8-12]. The International Society for Thrombosis and Hemostasis has defined DIC syndrome is an acquired syndrome characterized by intravascular activation of coagulation with loss of localization arising from various causes [13]. The data on the possibility of using screening coagulation parameters for the assessment and predicting early mortality in patients with simple and strangulated SBO are contradictory nowadays.

THE AIM

The study aimed to evaluate the possibility of using screening markers of coagulation to the assessment of severity and predict short-term outcomes in patients with small bowel obstruction.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

A two-center retrospective study at the Kharkiv National Medical University, Ukraine, included 71 patients over 18 and less than 60 years of age who was admitted to the ICU during the immediate postoperative phase. The study was conducted a bit more than two years, from September 1, 2019, to November 30, 2021, and with the approval of the University Ethics Committee (the protocol of Ethics Committee No. 5-21 (0116u00499) from October 25, 2021).

Inclusion criteria: the study included men and women who were admitted to the hospital with SBO.

Exclusion criteria: patients with mechanical large bowel obstruction; inguinal hernia; early postoperative SBO occurring less than 30 days after the abdominal operation; patients with a known history of ascites; comorbidity with acute myocardial infarction and stroke; post-resuscitation illness due to stopping effective blood circulation and a refractory shock; pregnancy; cancer in anamnesis.

PATIENTS AND COLLECTED VARIABLES

All of these patients were undergoing surgery. The primary outcome measure was death and it was defined as all-cause in-hospital mortality 30 days after surgery. Patients were divided into two groups: in the 1st included those with a positive outcome (survivors, n = 64), and in the 2nd those with adverse outcomes (non-survivors, n=7). The following data were collected: age, gender, body mass index (BMI), laboratory data with the determination of coagulation status in the volume of screening coagulogram; the severity was calculated for each patient: Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA) and the International Society on Thrombosis and Hemostasis (ISTH) Criteria for Disseminated Intravascular Coagulation (DIC) scores (ISTH for DIC), and the nature of complications and results (short-term mortality up to 3 days and during 30 days). There were two clinical observations and assessments during surgery: patients with reversible small bowel ischemia and patients with irreversible small bowel ischemia. Complications after surgery were graded according to the Clavien-Dindo classification [14].

STATISTICAL ANALYSIS

Comparison of demographic and clinical data of the patients (survivors and non-survivors) was accomplished by employing Mann–Whitney test and $\chi 2$ test for quantitative variables (1st step). Continuous data were presented as Me (Q1; Q3), where Me is the median, Q1 and Q3 is the interquartile range (IQR). Zero hypotheses (H0) in statistical tests were rejected at a significance level of $p \geq .05$. The capability of each prognostic index and biological markers to predict mortality was analyzed by the receiver operating characteristic (ROC) curve. The area under the ROC curve (AUC) and the respective confidence interval (CI) was used as a measure of the overall index accuracy by the selection of parameters in the 1st step. The areas under the ROC curve were determined for each score and each biomarker, and the significance of the differences between them was assessed and taken into account its 95% confidence interval. The prognostic efficacy of the models was assessed by discrimination based on the AUC index: the efficacy of the model was considered limited at AUC $\geq$ 0.70; was considered a good model at AUC $\geq$ 0.80, and excellent at AUC $\geq$ 0.90. Statistical analyses were performed using the software STATISTICA 13.3 EN.

RESULTS

PATIENTS, OPERATING DATA AND POSTOPERATIVE COMPLICATIONS

During the study period, 71 patients underwent emergency surgery for SBO. Of these, 7 patients (9.9%) died in the hospital postoperatively (Figure 1). From 28 (39.4%) patients with strangulated small bowel obstruction (SSBO), the adhesive disease was the primary etiology in patients (71.4%), and the volvulus was the second (28.6%).

Patient characteristics are shown in Table I. The groups that were studied did not have significant differences in gender. In survivors of patients were of onset of surgery more likely than in non-survivors, and in patients who survived were of onset of resuscitation and its duration before surgery also more early. Only the laparoscopy approach has been in 12.5%, only the laparotomy in 78.9%, and the hybrid in 9.9% of patients.

LABORATORY STUDIES

BASELINE CHARACTERISTICS

The results of the distribution of patients by the changes of laboratory data and severity depending on the outcome are presented in Table II. There was no significant correlation between the individual parameters of the integration tests and the indicators of the screening coagulogram before surgery in two groups of patients.

The data of patients with increased levels of D-dimer, CRP, and lactate in patients before surgery in a comparative aspect is presented in Figure 2. As it was shown, only the level of blood lactate significantly differed in patients with non-SSBO and SSBO before surgery (P = 0.012).

ROC CURVES ANALYSIS OF BIOMARKERS FOR PREDICTING EARLY MORTALITY

The indicators were selected that were credible and belonged to the category of good and excellent models before and after surgery as shown in Table III. The AUC of lactate before surgery was 0.805 (P = 0.000), and AUC of SBP was 0.808 (P = 0.000). At the same time, the APACHE II and SOFA scores were reliable for assessing the severity of patients, however, the SOFA score turned out to be more

Table I. Patients, operating data and postoperative complications

Indicators	Outcomes		P value
	Survivors (n = 64)	Non-survivors (n = 7)	
Ages, Me [IQR]	54 [18-58]	56 [43-60]	.132
Sex:			
- male	28 (43.8%)	2 (28.6%)	.078
- female	36 (56.2%)	5 (71.4%)	
BMI, kg/m2, Me [IQR]	24 [22-29]	31 [24-35]	.000
Hypotension at diagnosis (SBP < 90 mm Hg), (%) **			
IV fluid administration before surgery, (%) **	7 (10.9%)	5 (71.4%)	.014
Vasopressor before surgery, (%) **	64 (100%)	7 (100%)	.778
Onset to surgery, (min) *	5 (7.8%)	7 (100%)	.000
Me [IQR]	80 [60–150]	90 [60-125]	.449
Surgical approach, (%) **:			
Laparoscopy only	8 (12.5%)	–	.014
Laparotomy only	50 (78.1%)	6 (85.7%)	
Laparoscopy + Laparotomy	6 (9.4%)	1 (14.3%)	
Type of surgery, (%) **:			
Adhesiolysis, drainage	42 (65.6%)	1 (14.3%)	.000
Resection of the small intestine, anastomosis	19 (29.7%)	5 (71.4%)	
Resection of the small intestine, stoma formation	3 (4.7%)	1 (14.3%)	
Time of surgery (min), Me [IQR]	148 [40-170]	184 [110-190]	.016
Intraoperative fluid administration, (%) **	64 (100%)	7 (100%)	.078
Amount of fluid during surgery, (L) Me [IQR]	2.4 [1.8–2.8]	3.0 [1.5-3.2]	.011
Intra-abdominal complications, (%)**	12 (18.8%)	2 (28.6%)	.987
Re-laparotomy, (%)**	1 (1.6%)	2 (28.6%)	.041
Hospital length of stay (days), Me [IQR]	11 [9–23]	3 [1-5]	.000
Clavien-Dindo classification:		NA	
I		31 (43.7%)	
II		15 (21.1%)	
IIIa		7 (9.9%)	
IIIb		3 (4.2%)	
IVa		4 (5.6%)	
IVb		3 (4.2%)	

Note: * The time of surgery was defined as the time of starting surgery; ** Statistically significant difference as determined by χ2—criteria; P - Mann-Whitney test; NA - not applicable; BMI - body mass index.

significant before surgery: the AUC of its was 0.855 (P = 0.000).

At 72 hours after surgery, the predictive value of D-dimer, antithrombin, lactate levels, CRP, SBP, APACHE II and SOFA was also assessed using the ROC method (Table III). The area under the ROC curve (AUC) of D-dimer was 0.812 (P = 0.000); AUC of antithrombin was 0.815 (P = 0.000); AUC of lactate was 0.884 (P = 0.000); AUC of CRP was 0.833 (P = 0.000); AUC of SBP was 0.856 (P = 0.000). As it was shown by this research data, the APACHE II (AUC = 0.802) and SOFA (AUC = 0.844) scores were also reliable for assessing the severity of patients after surgery (P = 0.000).

Despite the small number of observations, the predictive value of ROC curves significantly differed and was more significant for the SOFA and APACHE II scores before surgery (Figure 3) compared to the level of D - dimer (AUC = 0.673) and the ISTH Criteria for DIC score

Table II. Laboratory data and scores of severity in patients depending on the outcome

Indicators	Before surgery		P	Via 72 hours		P value
	Survivors (n = 64)	Non-survivors (n = 7)		Survivors (n=64)	Non-survivors (n=7)	
Platelet (10^9/L), Me [IQR]	277 [210-401]	253 [203-309]	.055	267 [223-311]	211 [178-215]	.000
D-dimer (ng/mL), Me [IQR]	214 [112–324]	241 [213–378]	.268	212 [111-267]	265 [221-412]	.000
Fibrinogen (g/L), Me [IQR]	4 [3.1-5.4]	4.5 [3.8-6.8]	.141	4.7 [4.0-6.2]	5.8 [4.6-6.8]	.006
APTT (sec), Me [IQR]	31.5 [25-43]	33.5 [21-46]	.315	32 [22-44]	36.5 [24-48]	.350
INR (%), Me [IQR]	89 [65-99]	77 [64-82]	.176	85 [72-104]	73 [56-82]	.032
Antithrombin, (%)	80 [65-95]	68 [42-78]	.058	74 [62-91]	56 [40-74]	.003
Lactate (mmol/l), Me [IQR]	2.1 [1.8-2.8]	3.2 [2.6-5.1]	.000	2.2 [1.9-3.6]	4.8 [3.2-8.3]	.000
CRP (mg/l), Me [IQR]	86 [76-153]	183 [141-211]	.000	92 [82-162]	201 [160-213]	.000
SBP (mm Hg), Me [IQR]	115 [100-160]	80 [70-100]	.000	130 [90-155]	65 [60-100]	.000
APACHE II score (points), Me [IQR]	11 [9-16]	21 [15-28]	.000	14 [6-15]	26 [12-32]	.000
SOFA score (points), Me [IQR]	4 [3-9]	8 [5-11]	.000	9 [3-9]	14 [5-12]	.000
ISTH Criteria for DIC (points), Me [IQR]	0 [0-2]	1 [0-3]	.169	1 [0-2]	2 [1-4]	.006

Note: P - Mann-Whitney test; NA - not applicable; CRP - C reactive protein; APTT - Activated Partial Thromboplastin Time; INR - International Normalised Ratio; SBP – systolic blood pressure; APACHE II - Acute Physiology And Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment; ISTH for DIC – International Society on Thrombosis and Hemostasis Criteria for Disseminated Intravascular Coagulation.

(AUC = 0.503). At 72 hours after surgery, such markers as SOFA and APACHE II scores, and also D-dimer studied had a good predictive ability (Figure 4).

DISCUSSION

As usual, the non-neoplastic mechan includes two forms like a simple (non-SSBO) and a strangulated obstruction (SSBO) with the following causes: adhesions (80 - 91%), volvulus (4-6%), intussusception (3-5%), bezoar (1.2-4%), gallstones (0.5-6%), Crohn's disease (0, 7-3%), foreign bodies (0.2-1%), others (0.5-3%). The development of SBO triggers a whole cascade of diverse pathological processes affecting all organs and systems. However, the central link in the development of obstruction is directly itself the small intestine, representing the primary and main source of endogenous intoxication and MODS [15].

It is known that SBO with/without small bowel entrapment, sepsis and other critical disorders in abdominal surgery are associated with coagulopathy ranging from hypercoagulability to acute disseminated intravascular coagulation (DIC) [16]. It was shown that the hemostasis-related parameters as a predictor of poor outcomes in ICU patients [17]. Wherein, D-dimer, protein C, protein S, von Willebrand factor and antithrombin indicators were most often mentioned in this context [18, 19].

In our study was shown that independent use standard (screening) of coagulogram parameters, such as fibrinogen, APTT, INR, D-dimer and ISTH Criteria for DIC in patients with SBO was not associated with the adverse short-term prognosis. Several studies have been published on the diagnostic significance of D-dimer in acute lesions of the small intestine [20], as it is well have known that damage to the vascular wall triggers a cascade of physiological reactions in the body leading to activation of endothelium with the formation of a blood clot. It consists of blood cells and fibrin protein but as a result of working of the fibrinolytic system, fibrin is split into smaller fragments, and a variety of fibrin degradation products of various molecular weights are formed, including D-dimers. For these reasons, the clot does not spread from the site of the vessel damage throughout the entire

Table III. Diagnostic value of D-dimer, Platelet, Antithrombin, Lactate, CRP, SBP, APP, APACHE II, and SOFA scores for in-hospital mortality

Variable	AUC	Optimal cutoff value	SE	95% CI	Sensitivity	Specificity
D-dimer, (ng/mL):						
Before surgery	0.673	215.5	0.0512	0.565-0.771	0.542	0.853
Via 72 hours	0.812	243.5	0.0846	0.773-0.854	0.864	0.857
Platelet, (10^9/L):	0.730	310	0.0587	0.622-0.754	0.500	1.0
Before surgery						
Via 72 hours	0.765	245	0.0723	0.632-0.789	0.612	0.876
Antithrombin, (%):	0.786	82.0	0.0762	0.689-0.832	0.560	0.789
Before surgery						
Via 72 hours	0.815	70.0	0.0876	0.694-0.843	0.621	0.794
Lactate, (mmol/L):	0.805	2.15	0.0643	0.711-0.854	0.600	0.821
Before surgery						
Via 72 hours	0.884	2.65	0.0721	0.743-0.934	0.782	0.911
CRP, (mg/l):	0.625	136.5	0.0512	0.511-0.721	0.486	0.877
Before surgery						
Via 72 hours	0.833	210.0	0.0867	0.754-0.897	0.667	0.905
SBP, (mm Hg):	0.808	112.0	0.0667	0.693-0.861	0.791	0.818
Before surgery						
Via 72 hours	0.856	135.0	0.0932	0.724-0.922	0.689	0.976
APACHE II score:						
Before surgery	0.785	13.0	0.0543	0.667-0.821	0.897	0.542
Via 72 hours	0.802	14.5	0.0789	0.676-0.864	0.95	0.857
SOFA score:						
Before surgery	0.855	5.5	0.0734	0.699-0.872	0.758	0.8
Via 72 hours	0.844	9.5	0.0812	0.682-0.883	0.840	0.966
ISTH Criteria for DIC:						
Before surgery	0.503	2.5	0.0512	0.412-0.546	0.45	0.071
Via 72 hours	0.762	1.5	0.0614	0.765-0.808	0.875	0.7

Note: CRP – C-reactive protein; SBP – systolic blood pressure; APACHE II - Acute Physiology And Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment; ISTH DIC - International Society on Thrombosis and Hemostasis Criteria for Disseminated Intravascular Coagulation.

vascular bed. The presence of fibrin degradation products reflects the simultaneous activation of both coagulation and fibrinolysis. The high D-dimer level is observed in different conditions such as ageing, inflammation, cancer, renal failure, etc. [21-23]. As it was shown by our study that an increase in the level of D-dimer level was observed in 11 (39.3%) of patients with SSBO before surgery, but it data was not reliable in comparison to patients with non-SSBO. Naturally, the issues may arise about the objectives of the study using these markers of coagulation in patients, especially since the laboratory diagnostic data do not play a significant role in establishing the fact of obstruction and strangulation of the small intestine? Since the biomarkers of coagulability such as fibrinogen, APTT, INR, D-dimer and ISTH Criteria for DIC, were not shown as specific enough for the diagnosis of obstruction and strangulation of the small intestine before surgery in our study, and they had been also useless for determining the need for surgery in any particular cases, these markers had used to determine the presence and severity of metabolic disorders in all of the patients.

In addition, for these purposes, we used commonly measured markers such as C-reactive protein (CRP), lactate level, systolic blood pressure, scores of SOFA and APACHE II [24, 25]. It should be pointed out that, even in the case of intestinal ischemia, many studies did not find significant differences in these indicators between patients requiring conservative treatment and those requiring surgery, making these markers a little informative for distinguishing between these categories of patients before surgery. Nevertheless, attempts to use indicators that are sensitive for diagnostics continue and one of these sensitive indicators is L-lactate which is the end product of anaerobic glycolysis, in that time while D-lactate is mainly produced by the intestinal bacteria. Since cells of people contain only an isomer lactate dehydrogenase that is synthesized in humans, the content of L-lactate is increasing during tissue hypoperfusion and cell hypoxia [26]. In 2020, M. Şahin

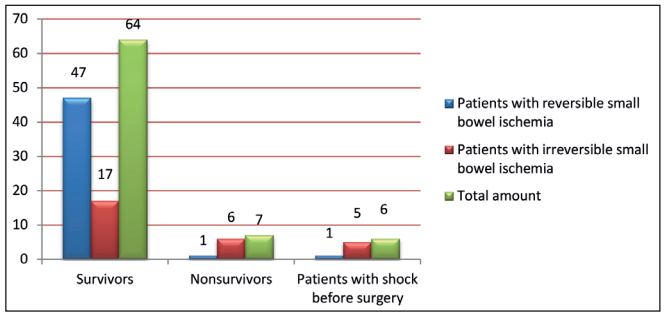

Fig. 1. Distribution of patients with strangulation of the small intestine.

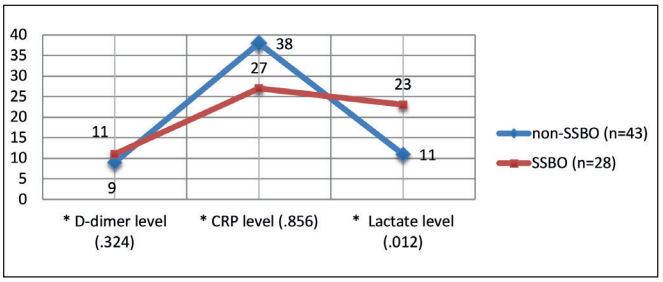

Fig. 2. Elevated preoperative D-dimer, CRP, and lactate levels in patients with non-SSBO and SSBO (* – the comparison was according to the χ2 test).

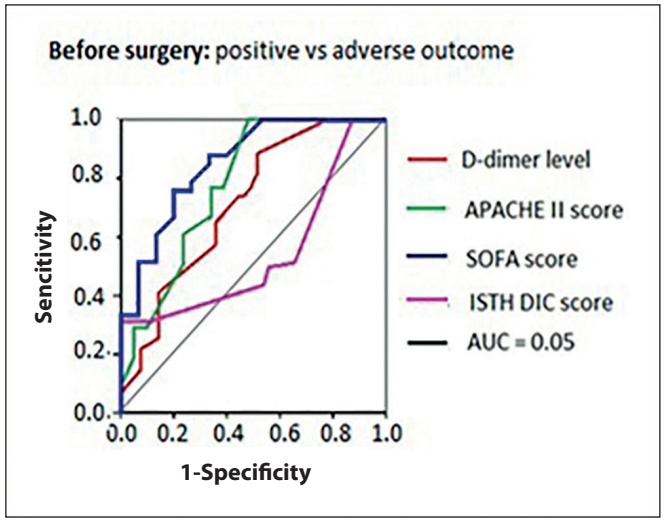

Fig. 3. Performance of prediction models as assessed by the AUC for death before surgery.

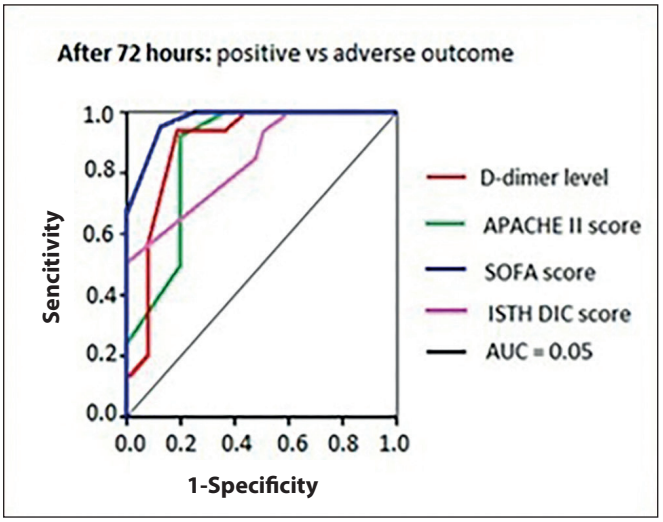

Fig. 4. Performance of prediction models as assessed by the AUC for death via 72 hours of surgery.

et al. noted that the presence of an intestinal obstruction in hernias and high serum lactate levels might indicate a poor prognosis and the need for resection of the small intestine in patients with strangulation during the preoperative assessment [27]. In our study, serum lactate before surgery (optimal cutoff value was 2.15 mmol/L) had a good predictive value (AUC = 0.805) with low sensitivity (60%) and high specificity (82.1%) (P = 0.000) as an indicator of prognosis for short-term mortality before surgery. This indicator also significantly differed in patients with SSBO and non-SSBO before surgery (P = 0.012).

To be sum up, the careful monitoring of hypotension and biochemical changes associated with metabolic disturbances in patients with SBO can contribute to the early prevention of complications that might occur after emergency surgery in these patients.

Our research has several limitations. First of all, this was a retrospective study, our data was based on the medical records of patients who were treated in only two hospitals, and we could not completely avoid some bias in the selection of data. Secondly, due to the relatively small clinical sample, the results obtained require further verification, which will make it possible to more clearly establish the set of preoperative data on the presence of infringement of the small intestine before the operation and the timing and type of surgical interventions in our opinion.

CONCLUSIONS

The study confirmed that three clinical and laboratory parameters are factors associated with short-term outcomes in SBO before surgery (lactate level, systolic blood pressure, and SOFA score) and seven parameters during 72 hours after surgery (D-dimer, antithrombin, lactate, CRP levels, systolic blood pressure, APACHE II, and SOFA scores). Screening tests of coagulation did not affect prognosis before emergency surgery in adults, and although in patients with SBO some of the fibrin degradation products were sensitive markers for short-time mortality due 72 hours after surgery, monitoring of indicators of coagulogram can be used as an estimate the potential risk of developing DIC and monitor an initiated conservative therapy. Taking into account the complexity and ambiguity of the results obtained, and the inconsistency of other data, it is necessary to continue studying the potential factors affecting the risk of bowel strangulation in SBO and the prognosis of mortality in different age groups.

ABBREVIATIONS

APACHE II: Acute Physiology and Chronic Health Evaluation; APTT: activated partial thromboplastin time; AUC: area under the ROC curve; BMI: body mass index; CRP: C-reactive protein; CT: computed tomography; DIC: dis-

seminated intravascular coagulation; ICU: intensive care units; IQR: interquartile range; ISTH: International Society on Thrombosis and Hemostasis; IV: intravenous; MODS: multiple organ failure syndrome; non-SSBO: non-strangulated small bowel obstruction; INR: International Normalised Ratio; SBO: small bowel obstruction; SBP: systolic blood pressure; SOFA: Sepsis-related Organ Failure Assessment; SSBO: strangulated small bowel obstruction.

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ORIGINAL ARTICLE

THE PREVALENCE OF RUBBER DAM AMONG DENTISTS IN DIFFERENT COUNTRIES

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ABSTRACT

The aim: To assess the actual prevalence of rubber dam usage among general dentists.

Materials and methods: Surveyed participants were offered a questionnaire containing 14 questions about gender, country of origin, clinical experience, time and place of acquisition of skills of rubber dam, as well as the frequency of its usage.

Results: 30.69% of dentists always use rubber dams for direct restorations; 74.26% always use rubber dams during root canals treatment; 36.3% always use rubber dam for bonding indirect restorations.

Conclusions: The prevalence of rubber usage among general dentists shows positive growth dynamics, but the frequency is still considered insufficient.

KEY WORDS: rubber dams, dental general practice, endodontics, school dentistry, dental research

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INTRODUCTION

Undoubtedly, the rubber dam is an adjunct that has proven its effectiveness in dental practice, in particular in preventing the spread of cross-infections, swallowing instruments, as well as a tool that indirectly improves the quality and success rate of restorative and endodontic manipulations. Despite the fact that many researchers find it very useful [1-4] and recomendations of numerous associations to apply it in everyday clinical practice [5-7], the prevalence of its usage remains far from desirable, according to some sources [4,8–10]. Alarming statistics are observed among general dentists, who occupy the most massive pool among all dentists around the world.

THE AIM

The aim of the study was to assess the actual prevalence of rubber dam usage among general dentists. Also, identify the relationship between the prevalence of rubber dam and clinical experience, the place of practice of the interviewed dentists as well as to clarify the most challenging issues in rubber dam application procedure.

MATERIALS AND METHODS

In order to achieve the objectives of the study, a survey method based on a questionnaire was chosen. To attract an international audience, it was decided to offer a questionnaire through two popular dental communities on the social network (Facebook) with 29.0 and 86.4 thousand participants, respectively. The questionnaire contained 14 questions about gender, country of origin, clinical experience, time and place of acquisition of skills in the use of the rubber dam, as well as the frequency of its application and was created in Google Forms.

Only general practice dentists were filtered from the general pool of answers. The rest of the participators were not considered. Respondents who did not provide demographic and reference data were excluded from further research.

Microsoft Statistical Excel 2016 and IBM SPSS Statistics 22 were used for statistical processing of the obtained data. The chi-square criterion was calculated to find the relationship between the rubber dam usage and various factors, the significance threshold was set at $p = 0.05$ [11].

RESULTS

A total of 165 dentists of various specialties completed the questionnaire. The cohort of general dentists was 64.85% - 107 people. 94.39% of this group was then considered (6 out of 107 people were excluded according to the policies of the study). A total of 57 men (56.44%) and 44 women (43.56%) participated. The geography of the study was rather wide. The largest number of responses were received from Ukraine 52 (51.49%) and France 37 (36.63%). Some separate forms came from Russia - 5 (4.95%) and Egypt, Czech Republic, Slovakia, Italy, Germany, Belgium, Georgia - 1 (0.99%) from each country.

The majority of respondents were practicing in large cities of more than 1 million inhabitants (29 - 28.71%) while 28 - 27.72% in cities from 250 thousand - 1 million population. A small part (14-13.86%) has had clinical practice in medium-sized cities of 100-250 ths.inhab. and 50 - 100 ths. inhab. (1-0.99%). The rest

Table I. Period of rubber dam implementation into practice and career duration

Implementation period	Clinical experience (persons)				
	≤5 years	6-15years	16-25 years	26-35 years	≥36 years
From the beginning of the career	38	19	1	0	1
With a certain delay	11	15	8	4	1
Do not apply rubber dam	2	0	1	0	0
Total	51	34	10	4	2
Grand total	101				

Table II. The interrelation between the origin of the rubber dam application skill and the period of its implementation into practice

Implementation moment	Origin of skills acquisition (persons)			
	University / dental school	Master class	Independent	No data
From the beginning of the career	30	9	20	0
With a certain delay	5	7	27	0
Do not apply rubber dam	1	0	1	1
Total	36	16	48	1
Grand total	101			

Table III. Frequency of rubber dam usage for direct restorations

Implementation moment	Frequency of usage (persons)				
	Always	Regularly	Occasionally	Never	No data
From the beginning of the career	21	27	10	1	0
With a certain delay	10	16	10	2	1
Do not apply rubber dam	0	0	1	2	0
Total	31	43	21	5	1
Grand total	101				

Table IV. Frequency of rubber dam usage for endodontic treatment

Implementation moment	Frequency of usage (persons)				
	Always	Regularly	Occasionally	Never	No data
From the beginning of the career	47	8	3	0	1
With a certain delay	28	5	3	2	1
Do not apply rubber dam	0	0	1	2	0
Total	75	13	7	4	2
Grand total	101				

were practicing in small settlements of 10-50 ths. inhab. (13 - 12.87%) and less than 10 thousand population (14 - 13.86%) (Table I).

Also, the vast majority of respondents (88 - 87.13%) were employed or self-employed in private practice clinics, while only 2 - 1.98% worked in public / municipal hospitals and 4 - 3.96% in dental schools / medical universities, 6 - 5, 94% of respondents practiced in several different places (Table I). In terms of clinical experience, the majority (51 - 50.49%) were young dentists with less than 5 years of experience, the second largest (34 - 33.66%) was a group of 6 to 15 years of experience. The group from 16 to 25 years of experience was represented by 10 - 9.90% while 4 - 3.96% and 2 - 1.98% of participants were from groups from 26 to 35 years and over 36 years of clinical practice, respectively.

The majority of respondents 48 - 47.52% replied about having acquired the rubber dam application skills alone or with the help of thier colleagues, 36 - 35.64% received the necessary knowledge while studying at university or dental school, and 16 - 15.84% said that they attended special master classes independently. The respondents that started using the rubber dam from the beginning of their career are represented by the group of 59 - 58.42%, while others (39 - 38.61%) started using it with a delay. There were very few dentists who claimed not using rubber dam at all (3 - 2.97%). Among all participants, 74-73.27% of respondents were mostly satisfied with their manual skills of rubber dam usage and 16 - 15.84% were completely satisfied, while 8 - 7.92% were mostly dissatisfied, 1 - 0.99% were completely dissatisfied and 2 - 1.98% could give a distinct reply.

The frequency of rubber dam usage for direct teeth resto-

rations was as follows: 31 - 30.69% of doctors indicated its mandatory application, while 43 - 42.57% said claimed to use it in most cases, 21 - 20.79% indicated its irregular usage, 5 - 4.95% do not use rubber dam for direct restorations and 1 - 0.99% of respondents do not perform direct restorations at all.

As for the rubber dam application for endodontic treatment, the situation was much better, i.e. 75 - 74.26% stated its mandatory usage, 13 - 12.87% were using it in most cases, 7 - 6.93% indicated periodic usage and only 4 - 3.96% did not use it for root canal treatment, while 2 - 1.98% of general dentists do not perform endodontic treatment.

In contrast to the previous case, the prevalence of rubber dam usage was much worse for indirect restoration bonding: only 29-27.7% of respondents stated to apply it obligatory, 23-22.77% - in most cases, 18-17.72% - sometimes and 10 - 9.90% never use rubber dams for this procedure. In addition, 16 - 15.84% of respondents do not perform indirect restorations at all, and 5 - 4.95% failed to answer distinctly.

According to the questionnaire, only 17 - 16.83% of respondents do not have difficulties with the rubber dam application, while the majority (71 - 70.29%) report about facing various technical problems, e.g. poor clamp retentions, latex curtain tears, problems with rubber dam inversion, etc. Quite a significant part (32 - 31.68%) reports numerous difficulties, e.g. technical problems and reluctance of the patient, economic reasons and lack of time, etc. After a more profound clarification of the technical difficulties that general practitioners regularly face with, it was found that the most common and recurring difficulty is poor clamp retention (73 - 72.28%). Regular fluid leakage from under the rubber dam edges is reported by 49 - 48.51% of respondents. However, 61 - 60.39% of respondents regularly face more than one difficulty.

Statistical checkup revealed a significant difference between French and Ukrainian general dentists in the period of introduction of rubber dam into their clinical practice. Namely, 83.8% of French versus 40.4% of Ukrainian general dentists claimed to start using rubber dams from the very beginning of clinical practice $\chi2(18, N = 101) = 55.82, p = .012$. The difference is also noticeable when comparing the answers of Ukrainian and French doctors to the question of origin of rubber dam application skills, i.e. 3.8% of Ukrainians report acquiring these skills in medical school classes against 81.1% of French respondents; $\chi2$ (27, N = 101) = 71.33 $p < .05$. Another significant difference between Ukrainian and French general dentists was also found in the parameter of mandatory use of rubber dam for bonding of indirect restorations, which was 20.7% against 65.5%, respectively; $\chi2$ (45, N = 101) = 79.50, p = .001.

It is noteworthy that the majority (74.5%) of young doctors (less than 5 years of work experience) use rubber dam from the very beginning of their career compared to the general cohort (58.4%), $\chi2$ (8, N = 101) = 24, 19, p = .002 (Table I).

Another notable parameter is the relationship between the time of acquisition of skills and their implementation into clinical practice: 83.3% of general dentists who claimed to achieve the skill at the university, began its instant implementation, comparing to those who attended special courses (56, 3%), and to those who independently achived the skill (41.7%), $\chi2$ (6, N = 101) = 49.09, $p < .05$ (Table II).

Regarding satisfaction rate with manual skills, it should be noted that the rate of absolutely satisfaction with the rubber dam skills was the highest in the group of those who reported using a rubber dam from the beginning of their career (75%); $\chi2$ (8, N = 101) = 25.69, p = .001.

The percentage of practitioners who obligatory use rubber dam for direct restorations is 35.6% Most respondents have been using it since the beginning of their careers (67.7%) compared to those who acquired the skill with a delay (32, 3 %); $\chi2$ (8, N = 101) = 30.17, p = .034 (Table III).

The prevalence of mandatory rubber dam usage during endodontic treatment was 74.3%. Also, the percentage is significantly higher among those who has been using it since the beginning of clinical practice (62.7%) compared to those who has been applying it with a delay (37.3%); $\chi2$ (8, N = 101) = 38.64, p = .011 (Table IV).

Concerning the rubber dam usage for indirect restorations bonding only 36.3% of respondents apply it for this type of procedure. At the same time, 86.2% of this cohort have used it since the beginning of their careers, $\chi2$ (6, N = 80) = 34.43, $p < .05$.

DISCUSSION

The number of participants was lower than in the survey performed in the Czech Republic - 450 [12] or Saudi Arabia - 193 [19], but very close to those conducted in Turkey - 143 [18], India - 101 [17] and Nigeria - 100 [9] and even more than some others. This amount was sufficient for regular statistical analysis.

Although the survey covered 9 countries, we explain the origin of majority of participants from some particular countries, such as Ukraine and France, with the particular audience of social networking groups used for the questionnaire placement, although they were international and available for dentists from around the world. In addition, the predominance of participants with little clinical experience (less than 5 years) can also be explained by the location of the questionnaire, as young people are generally considered to be more active in social networks than more mature people.

It is observed that dentists who have acquired the rubber dam usage skills during undergraduate education use it more regularly, which is consistent with the results of Kapitán & Šustová, 2011 [12]. The level of satisfaction and prevalence of rubber dam use is also higher among early users [12,13].

However, there is a disparity in skills acquisition between different countries (e.g. Ukraine and France). Despite the fact that the difference is furtherly eliminated by gaining skills at additional training and master classes, the reasons of undertraining of Ukrainian dentists at the undergraduate stage is a field of interest.

Mandatory rubber dam usage is more common for endodontic treatment (74.3%) rather than for direct or indirect restorations (35.6% and 36.3%, respectively). The prevalence of its mandatory application in endodontic procedures, which we received in our study, was much higher than in many other recent studies, for example: Palmer et al., 2009 - 30.3% [14], Koch et al., 2009 - 67% [15], Hill & Rubel, 2008 - 58% [16], Kapitán & Šustová, 2011 - 26%, Jena et al., 2014 - 34.4% [17].

We also share the opinion of Kapitán & Šustová, 2011 [12] that the prevalence of rubber dam usage could theoretically be

lower if older dentists were more involved. It is also should be mentioned that in some countries the training for the rubber dam application has been included as the mandatory part of undergraduate dental training relatively recently. Also, the questionnaire was posted in Facebook communities which audience consists mainly of young practitioners and those who are more committed to their profession.

CONCLUSIONS

Thus, we can conclude that:

- The prevalence of rubber dam usage among general dentists shows positive growth dynamics, but the frequency is still considered insufficient.
- Mandatory rubber dam application is more common for root canal treatments than for direct or indirect restorations.
- Undergraduate studies show a more positive effect on the effectiveness of further implementation of the rubber dam in the daily practice of general dentists than the acquisition of such skills at a later stage.
- Study programs of medical universities and dental schools in some countries require the revision and introduction of additional training hours for mastering rubber dam application skills.

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ORIGINAL ARTICLE

EXPRESSION OF SYNAPTOFYSIN AND VEGF IN THE SENSOMOTOR CORTEX DURING THE CAROTID ARTERY LIGATION, THE BRAIN ANTIGEN SENSITIZATION AND THEIR COMBINATIONS

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ABSTRACT

The aim: To study changes of the expression of synaptophysin (Syn) and vascular endothelial growth factor (VEGF) in neurons of the sensorimotor cortex (SMC) to reveal after unilateral ligation of the carotid artery, sensitization with brain antigen and their combination.

Materials and methods: Experimental animals – Wistar rats (260-290 g). Experimental models: mobilization of the left common carotid artery, ligation of the indicated artery, sensitization with cerebral antigen, combination of sensitization with cerebral antigen and ligation of the carotid artery. Methods: immunohistochemistry, quantitative densitometric assessment.

Results: Dyscirculatory disorders of cerebral blood supply during unilateral mobilization or ligation of the common carotid artery, sensitization with cerebral antigen lead in rats to a transient decrease in synaptophysin expression and phase changes in VEGF expression in the SMC from the lesion side. These changes occur in the absence of morphological changes in the cerebral cortex.

Conclusions: The absence of morphological changes in the SMC in the short term (10-30 days) after minor trauma to the common carotid artery (separation from the bed and n.vagus) or its ligation is accompanied by a transient decrease in Syn expression and some increase in VEGF, which may reflect a violation of synaptic function and the general metabolic activity of neurons. Sensitization with a brain antigen, leading to an increase in the level of anti-brain antibodies and immune complexes in the blood of rats, can act as an independent damaging factor for the brain, and also potentiates and prolongs changes caused by impaired blood circulation.

KEY WORDS: Sensorimotor Cortex, Cerebrovascular Trauma, Neuroimmunomodulation, Synaptophysin, Vascular Endothelial Growth Factors

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INTRODUCTION

Decreased cerebral circulation is a risk factor for the development of neurodegeneration, cognitive impairment and dementia [1]. The most significant neurological disorders in cerebral hypoperfusion are associated with damage to the myelinated nerve fibers (myelin sheaths) of the white matter [2]. Under these conditions, neuronal death was also observed in the hippocampus [3] and in the cerebral cortex [4, 5]. It should also be noted that different parts of the brain respond to hypoperfusion in different ways and at different time intervals [4].

The overwhelming number of results on pathogenetic mechanisms, cellular reactions in the brain during its hypoperfusion, obtained in experimental models. In most models, severe cerebral hypoperfusion was modeled, which was most often reproduced by two-way simultaneous ligation of the carotid arteries [6]. Less severe consequences were observed using the model with delayed ligation of the second carotid artery [3]. Only a few works were performed on "soft" modes of hypoperfusion, which were based on bilateral stenosis of the carotid arteries [6, 7] or unilateral ligation of the carotid artery [4].

Under physiological conditions, the nervous tissue of the brain is separated from the immune system by the blood-brain barrier. However, in the blood of 5 to 92% of people, anti-brain antibodies are detected, which can damage the brain, initiate or intensify neurological manifestations [8].

We [9] have shown changes in the specific density of cells in the SMC composition in rats in the long term after ligation of the right carotid artery (3 months). This circumstance was the reason for conducting a quantitative immunohistochemical study to identify changes in the state of neurons in the cerebral cortex during hypoperfusion, primarily in the early stages after circulatory disorders, as well as during preliminary sensitization of animals with brain antigen.

THE AIM

The aim is to identify changes in the expression of synaptophysin (Syn) and vascular endothelial growth factor (VEGF) in neurons of the sensorimotor cortex (SMC) during unilateral ligation of the carotid artery, sensitization with brain antigen and their combination.

MATERIALS AND METHODS

In the experiment, we used 185 males white Wistar rats (weight 260-290 g), which were kept under standard conditions and a diet.

The entire sampling of rats was randomly divided into six groups. The intact control group (C; $n = 10$) allowed us to obtain a relatively normal sampling for comparison; it included intact animals subjected to no interventions. In the PO group ($n = 35$), rats were subjected to a sham operation (opening of the left common carotid artery, mobilization of the latter with no ligation, and subsequent suturing of the wound). In this group, we tried to estimate possible effects of the above-described surgical manipulations with no influence on brain circulation. The LAC group ($n = 35$) included animals with opening, mobilization, and *ligation* of the left common carotid artery with a silk ligature; thus, hypoperfusion of the brain was the main factor affecting the respective animals. In the Cs and POs groups ($n = 35$ in both cases), intact and sham-operated rats were *sensitized* by injection of cerebral antigen (see below); an isolated action of this antigen was considered the main experimental factor. Finally, rats of the LACs group ($n = 35$) were subjected to ligation of the carotid artery and preliminary sensitization; thus, those animals were subjected to a combined action of the respective factors. Sensitization was performed in animals of the ShS and LACs groups 10–12 days before surgery; a 20% water–salt extract of the homologous brain tissue (antigen; protein content 0.33–0.50 mg/ml according to Lowry) was injected subcutaneously in the amounts 0.5, 1.0, and 1.5 ml on days 10–12 respectively before surgery [10]. In the CS group, injections of the extract were performed synchronously and in the same mode as those in the POs and LACs groups

Surgical manipulations were carried out under thiopental anesthesia (50 mg/kg, i.p.). Euthanasia of the animals was performed by injection of an overdose of thiopental (200 mg/kg).

Brain samplings were taken from euthanized animals on days 1, 3, 10, 30, and 90 after surgery (days 12, 15, 22, 42, and 102 respectively after initiation of sensitization). The rat skull was quickly opened, and the brain was removed and cut into three parts by frontal incisions. The middle brain portion was fixed in chilled (4°C) 10% buffered formalin (pH 7.4, 24 h). Samples were compacted in Paraplast, 4 μm thick frontal sections were prepared and stained with azure II-eosin (to estimate a general condition of the cerebral cortex), and immunohistochemical reactions were performed according to the protocols of the antibody manufacturers. The following primary antibodies were used: mouse monoclonal antibody against Syn (Synaptophysin Ab-2, Clone SYP02; Thermo Fisher Scientific, USA), at a dilution of 1: 100; rabbit polyclonal antibody against VEGF (Vascular Endothelial Growth Factor Ab-1 (RB-222), VEGF165 immunogen; Thermo Fisher Scientific, USA), dilution 1: 200. The reaction products were visualized using a diaminobenzidine-based detection system (EnVision FLEX; Dako, Glostrup, Denmark).

Incubation of the sections with antibodies was performed at 24°C (with primary and secondary ones, for 20 and 10 min respectively). As positive controls, sections of the rat brain with a certain positive expression of the protein were used; for negative controls, all procedures were performed except use of primary antibodies. The sections for densitometric measurements were enclosed with coverglasses in a water-soluble medium (Dako Ultramount Aqueous Permanent Mounting Medium; Dako, Glostrup, Denmark). Other sections were additionally stained with Gill's hematoxylin and enclosed in a histological balsam.

The obtained preparations were examined under an Olympus BX51 microscope and photographed with an Olympus C3040ZOOM digital camera using the Olympus DP-Soft 3.2 software (Olympus, Tokyo, Japan). Densitometric measurements of the expression Syn and VEGF were performed on digital images with a ×400 magnification (1280x960 RGB pixels, photo illumination mode, standardized exposure); an image analysis system ImageJ 1.46 (Wayne Rasband, NIH, USA) was used. In each case, five test fields of images of the fifth layer of the left-hemisphere SMC were studied.

The obtained numerical data were processed by standard statistical methods with calculations of the arithmetic mean, standard deviation, and standard error of the mean. Exact Kolmogorov–Smirnov Test showed that all data of experimental measurements are not inconsistent with a normally distribution. Student's t test was used to assess the significance of intergroup differences; at $P < 0.05$, the latter were considered statistically significant.

RESULTS

In the rats of the control group, the SMC had a normal structure. IHC showed the presence of high / moderate expression of Syn in the neuropil and the absence in the body of neurons and glial cells. In the neuropil, small granules were usually marked in the form of clusters of different densities. Quite often, it was possible to observe chromogen granules located linearly on the surface of the initial section of the apical dendrite of pyramidal neurons of the 5th layer of the SMC. Less often, similar granules could be found on the surface of the bodies of these neurons, and in individual neurons, they formed a kind of capsule.

In the control, VEGF had moderate to strong expression in the body of SMC neurons. In this case, the chromogen granules were more often located under the plasmalemma and, in smaller quantities, around the nucleus. Occasionally, a mark (usually faint) could be observed in the apical dendrites of pyramidal neurons. Moderate expression of VEGF was observed in the neuropil (in the form of small granules). A low expression of this factor was also found in the cytoplasm of a part of the bodies of glial cells.

Visual assessment of SMC after PO or LAC did not allow unambiguous identification of changes in its structure or Syn expression in it. There were also no changes in VEGF expression after PO. With LAC, there was a slight decrease in it in the perikaryon and neuropil after 10 days of the experiment, after which, on the 30th day, the peak increase in the expression of this factor looked contrasting.

Densitometric evaluation showed that after PO there was a slight decrease in Syn expression, which became statistically significant 3 and 10 days after the start of the

Table I. Expression of Syn in the neuropil and VEGF in neurons of the 5th layer of the sensorimotor cortex of the left hemisphere of the rat brain (optical density, conventional units). 1(12) - 90(102) – days after surgery (sensitization). Data is significantly different from: control C; pseudo-operations PO; control, sensitization Cs; Sensitization and pseudo-operations POs.

Group	Days	Syn				VEGF			
		M	δ	m	p	M	δ	m	p
Control		64,85	9,6	1,15	0,018	155,0	28,2	3,37	0,022
Pseudo-operations	1	65,2	12,4	2,09	0,032	159,9	28,1	4,75	0,03
	3	60,0^{C}	9,6	1,62	0,027	157,1	23,5	3,97	0,025
	10	59,3^{C}	13,9	2,35	0,04	161,5	24	4,06	0,025
	30	64,4	14,7	2,48	0,039	168,1^{C}	17,7	2,99	0,018
	90	64,1	11,2	1,89	0,029	152,7	23,5	3,97	0,026
Ligation of the carotid artery	1	59,7^{C,PO}	8,8	1,49	0,025	164,6	29,5	3,53	0,021
	3	56,7^{C}	7,8	1,32	0,023	145,6	42,7	5,1	0,035
	10	57,5^{C}	8,1	1,37	0,024	129,0^{C,PO}	43,8	5,23	0,041
	30	60,2	14,2	2,4	0,04	190,4^{C,PO}	37,4	4,47	0,023
	90	65,2	14,1	2,38	0,036	161,7	37,6	4,49	0,028
Control, sensitization	1(***12***)	60,7	12,34	2,08	0,034	139,6^{C}	24,84	4,2	0,03
	3(***15***)	60,56^{C}	11,29	1,91	0,031	133,4	22,17	3,75	0,028
	10(***22***)	59,98^{C}	12,8	2,16	0,036	123,8^{C}	16,43	2,78	0,022
	30(***42***)	60,8	11,1	1,88	0,031	128,9^{C}	21,84	3,69	0,029
	90(***102***)	63,6	11,71	1,88	0,03	146,2^{C}	21,24	3,59	0,024
Sensitization and pseudo-operations	1(***12***)	59,23^{C}	11,3	1,91	0,032	140,9^{C}	18,65	3,15	0,022
	3(***15***)	58,95^{C}	11,8	1,99	0,034	130,1^{C}	22,41	3,79	0,029
	10(***22***)	60,27	12,74	2,15	0,036	126,9^{C}	26,38	4,46	0,035
	30(***42***)	62,57	11,12	1,88	0,03	128,4^{C}	21,78	3,68	0,029
	90(***102***)	64,63	10,71	1,81	0,028	135,9^{C,Cs}	19,83	3,35	0,025
Sensitization and ligation of the carotid artery	1(***12***)	56,2^{C}	11,31	1,91	0,034	131,1^{C,POs}	23,72	4,01	0,031
	3(***15***)	56,61^{C}	11,2	1,89	0,033	124,8^{C}	25,16	4,25	0,034
	10(***22***)	59,27^{C}	12,74	2,15	0,036	125,0^{C}	19,46	3,294	0,026
	30(***42***)	60,87	10,92	1,84	0,03	124,5^{C}	22,89	3,869	0,031
	90(***102***)	65,23	11,71	1,99	0,03	140,4^{C}	23,21	3,92	0,028

experiment. After LAC in the period 1-3-10 days of the experiment, a more pronounced decrease in the expression of Syn compared to PO was determined, although the differences between these groups were statistically significant only after 1 day of the experiment. Subsequently, starting from the 30th day of the experiment, both with RO and LAC there was a gradual recovery of this indicator to the control values (Fig. 1).

After PO, VEGF expression in pyramidal neurons of the 5th layer of the SMC showed a tendency to increase, and after 30 days it slightly, but significantly exceeded the control values. In the late follow-up period (after 90 days), VEGF expression in the PO group was practically the same as in the control (Fig. 2).

In the LAC group, the specific density of VEGF-tags in neurons on the first day after surgery slightly, but significantly exceeded the initial values. Within 3-10 days, this indicator became significantly lower than the control value, but on day 30, it exceeded the latter by about 15%. After 3 months of observation, the optical density of the chromogen in the analyzed preparations was practically equal to the control value (Fig. 2).

Sensitization of rats to brain antigen resulted in diffuse alterative changes in the SMC. In individuals of the Cs group, 12, 15, and 22 days after the onset of sensitization in the SMC, moderate perivascular edema was observed. Neurons often had deformed contours and granular hypercondensated chromatophilic substance. Occasionally, focal chromatolysis phenomena were observed. Single degenerating hyperchromic and, less often, necrotic neurons were detected. Typical for this group in SMC were the phenomena of small-lipped degeneration with very small (dust-like) cavities. Over time, these phenomena became less pronounced. At the same time, there was an increase in the number of glial cells in the cerebral cortex, which at the end of the experiment (102 days after sensitization) could form small groups.

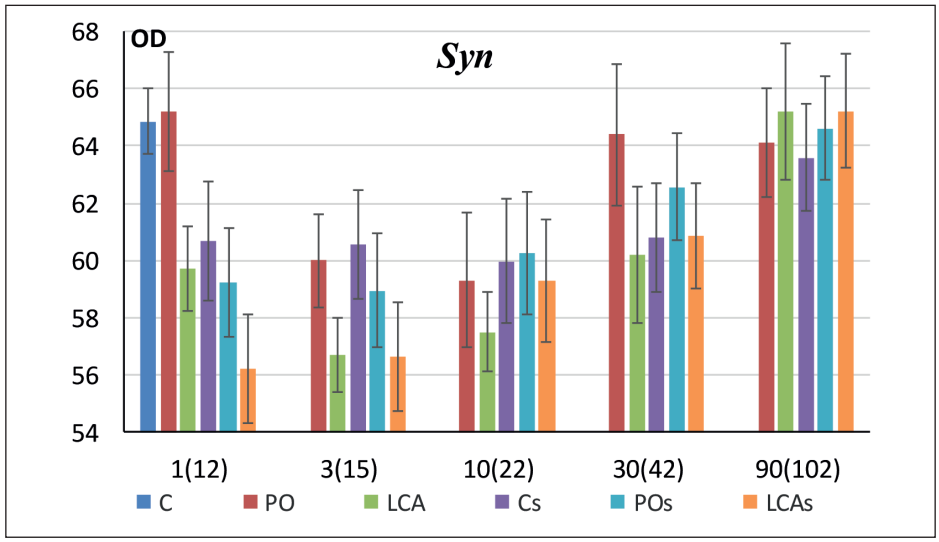

Fig. 1. Expression of Syn in the neuropil of the 5th layer of the sensorimotor cortex of the left hemisphere of the rat brain (M ± m). C – control, RO – pseudo-operations, LAC – ligation of the carotid artery, Cs – control, sensitization, POs – sensitization and PO, LACs – sensitization and LAC. OD – optical density (conventional units). 1(12) - 90(102) – days after surgery (sensitization).

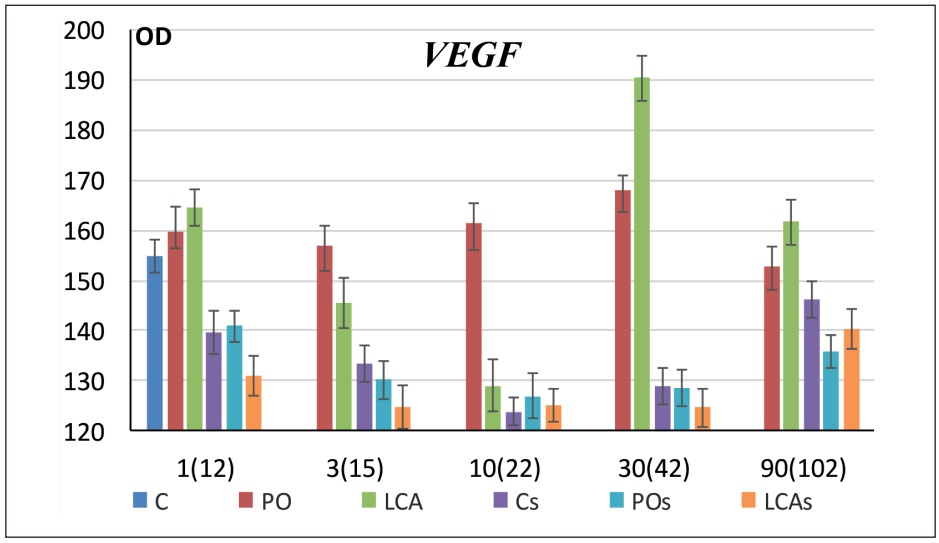

Fig. 2. Expression of VEGF in the cytoplasm of the neurons soma of the 5th layer of the sensorimotor cortex of the left hemisphere of the rat brain (M ± m). C – control, RO – pseudo-operation, LAC – carotid artery ligation, Cs – control, sensitization, POs – sensitization and RO, LACs – sensitization and LAC. OD – is the optical density (conventional units). 1(12) - 90(102) – day after surgery (sensitization).

In rats of the Cs group, 15 and 22 days after sensitization, the level of Syn expression in the SMC turned out to be significantly lower as compared to the intact animals. Moreover, in the neuropil, small areas were found where Syn expression was absent. Starting from day 42 after sensitization, a slow increase in the level of Syn expression in the sensorimotor cortex was observed, which on day 102 of the experiment did not differ from the control (Fig. 1).

Visually, in sensitized animals in SMC neurons, VEGF expression slightly decreased and its character changed. First, this was manifested by the loss of clarity of the contours of the perikaryons. At the early stages of observation (12, 15, and 22 days), vacuoles could be observed in them. The processes of neurons in these conditions were practically not visualized (Fig. 1).

In animals of the Cs group, 12, 15, 22 and 42 days after sensitization in the SMC, there was a statistically significant decrease in the level of VEGF expression in the cytoplasm of neurons. After that, it gradually increased, but even after 102 days, it did not reach the control values (Fig. 2).

In the rats of the POS and LACs groups, the general structure of the SMC visually practically did not differ from the Cs.

Densitometric assessment of Syn expression in SMC at POS showed its slight decrease on days 12 (1) and 15 (3) of the experiment as compared to Cs, but the differences were not statistically significant. With LACs, the decrease in the amount of Syn was even more pronounced. However, the level of differences in the numerical values of this indicator was not statistically significant for both POs and Cs. (Fig. 1).

Quantification of VEGF expression in animals with POs did not reveal statistically significant differences from Cs. With LACs, a more pronounced decrease in immunoreactivity to VEGF in SMC compared to Cs was noted, although these values did not reach statistical significance. (Fig. 2).

DISCUSSION

Approaching the assessment of the phenomena described above, it should be noted that the expression of Syn and VEGF in SMC neurons could, to a certain extent, be considered as markers reflecting their functional state. In this case, the content of Syn is directly related to the number of

synapses and synaptic function [11]. The signaling protein VEGF can be considered not only as a factor supporting adequate blood perfusion of brain tissue [12], but also as a factor in maintaining the overall level of neuronal activity [13]. The latter is associated with the presence of receptors on these cells, and its effects are an increase in anabolic processes and an increase in resistance to the action of negative factors [14].

As we have shown earlier [9], neither RO nor LAC directly leads to a noticeable change in SMC morphology. However, even such a minimal effect as mobilization of the common carotid artery without its subsequent ligation causes a significant transient decrease in Syn expression and a certain increase in VEGF expression, which at its peak becomes statistically significant. Ligation of the same carotid artery, which undoubtedly leads to dyscirculatory disorders, and, with a high probability, to hypoperfusion of the cerebral hemisphere, leads to more pronounced and prolonged changes in these markers, which, in principle, was expected [15].

The fairly rapid recovery to the initial level of Syn and VEGF expression in SMC under the conditions of our experiment can be explained by the use of healthy rats in the experiment, which are characterized by a high level of compensatory-restorative properties, small disturbances in hemomicrocirculation, and an acute nature of the pathological process. It would be a mistake to extrapolate this data to a person directly. This is due to the fact that in the overwhelming majority of cases in humans, blood circulation disorders in the brain appear gradually as a result of a chronic pathological process, and neurological manifestations occur when compensatory processes are disrupted [2]. Therefore, one should not expect a spontaneous reverse development of changes in the brain caused by hypoperfusion in a person, but a therapeutic intervention is necessary.

As we have shown earlier, sensitization with a brain antigen led to an increase in the level of anti-brain antibodies and circulating immune complexes in the blood [10]. At the same time, the number of reactively changed neurons in the SMC increased during the first month of observation and the number of astrocytes progressively increased after one and three months of observation [9]. Changes in Syn and VEGF expression in SMC with Cs were more approve than with PO and LAC. This suggests that the method of sensitization we used turned out to be more traumatic for the brain than PO and LAC.

Brain antigen sensitization generally potentiated and prolonged changes in the expression of Syn and VEGF caused by PO and LAC in the SMC. However, these changes were weakly expressed against the background of preliminary sensitization due to the relatively high level of its damaging effect on the brain.

CONCLUSIONS

The absence of morphological changes in the SMC in the short term (10-30 days) after minor trauma to the common carotid artery (separation from the bed and n. vagus) or its ligation is accompanied by a transient decrease in Syn expression and some increase in VEGF, which may reflect a violation of synaptic function and general metabolic activity of neurons. Sensitization with a brain antigen, leading to an increase in the level of anti-brain antibodies and immune complexes in the blood of rats, can act as an independent damaging factor for the brain, and also potentiates and prolongs changes caused by impaired blood circulation.

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ORIGINAL ARTICLE

CHRONIC PAIN AND PHYSICAL THERAPY IN CHILDREN WITH PARALYTIC SYNDROMES: ARE THERE ANY CHANGES DURING LOCKDOWN?

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ABSTRACT

The aim: To evaluate an influence of physical therapy on chronic pain in children with paralytic's syndrome and to maternal emotional status on lockdown time during the COVID-19 pandemic.

Materials and methods: Data from 96 children and their mothers (96 persons) were included in the study. On-site services of physical therapists before the pandemic (2018-2019) were received by 64 children and by 32 children during quarantine measures due to COVID-19 pandemic (2020). The age of the children ranged from 1 to 6 years, the median age was 3 years and 3 months.

Results: We note that there were more boys with paralytic syndromes. Among the leading paralytic syndromes, the most common was spastic tetraparesis. The frequency of children with level III–V motor disorders prevailed.

Conclusions: The authors consider that physical rehabilitation in children with paralytic syndromes reduces the incidence of moderate chronic pain and improves the emotional state of parents. But, these changes do not occur during the pandemic.

KEY WORDS: children, physical therapy, chronic pain, Covid-19 pandemic, paralytic's syndrome

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INTRODUCTION

Pain assessment and management are important components of pediatric care [1]. It affects various aspects of a child's and family's life, such as sleep, emotional state, social relationships, development and is usually caused by muscle spasticity, contractures, vertebral deformities, bedsores or skin macerations, hypersalivation and/or hyperproduction of bronchial secretions, tube feeding, tracheostomy, gastrostomy, colostomy, seizures, lesions of the central and peripheral nervous system [2]. The clinical assessment of pain is usually challenging in nonverbal children. Therefore, we use the pain assessment tools in a daily practice. [3, 4].

The current strategy for the treatment of chronic pain in children with paralytic syndromes and neurological lesions, defined by the WHO recommendations in 2012 [5]. These recommendations provide a multimodal approach of analgesia for the chronic pain treatment, including the widespread use of non-pharmacological methods and pharmacological therapy [6, 7].

Maternal emotional state is another important aspect that effects on the pain syndrome in children. The recent studies show that parents of children with paralytic syndromes experience chronic stress due to an incurable disease in a child. Therefore, not only the optimization of maternal physical and psychological health helps in stress management, but their involvement in active physical therapy may reduce anxiety, gives confidence, improves emotional state [8].

Significant lifestyle adjustments in the general population of children and adults have been made by the COVID-19 pandemic, which is associated with psychological problems [9]. Families of children with special needs remain a particularly vulnerable group. The American Academy of Pediatrics (AAP) provides information on caring for children and youth with special health care needs during the COVID-19 pandemic [10]. Health and/or education systems can provide services such as physical, occupational, speech, and other treatments as recommended by the AAP. These therapeutical approaches are crucial to children's development and should be continued during a pandemic. And the availability of services is necessary both in the case of personal visits to rehabilitation centers and in virtual therapy, as an alternative [11]. Even though the pandemic lasts about one year, the issue of rehabilitation of children with special needs remains predominantly unresolved [12].

HYPOTHESIS

1) physical therapy effectively influences on the chronic pain relief in children with paralytic syndromes; 2) physical therapy has a positive impact on the emotional status of mothers of children with special needs; 3) the COVID-19 pandemic changes the effects of physical therapy on chronic pain in children with paralytic syndromes and the emotional state of their parents during.

THE AIM

The aim of this work was to evaluate an influence of physical therapy on chronic pain in children with paralytic's syndrome and to maternal emotional status on lockdown time during the COVID-19 pandemic.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

This study is a non-interventional, descriptive, cross-sectional, single-center. The study procedures were conducted at intervals in 2018-2019 (before the COVID-19 pandemic) and in 2020 (during the COVID-19 pandemic and the implementation of quarantine measures). We provided the individual rehab plan to each child, depending on age, motor dysfunction and comorbidities. Pain assessment and questionnaires of children's parents with paralytic syndromes were performed at the beginning of rehabilitation and after 6 months (end-point).

ETHICAL APPROVAL

This study was approved by the Local Ethics Committee (Protocol № 9 from 16 October 2018), which was conducted with the involvement of underage patients and did not include measures that could harm their health and safety. Both parents of the patients were informed about the methods and scope of the study and agreed to their children participation in this research.

SAMPLING

Data from 96 children and their mothers (96 persons) were included in the study. On-site services of physical therapists before the pandemic (2018-2019) were received by 64 (66.6%) children and by 32 children (33.3%) during quarantine measures due to COVID-19 pandemic (2020). The age of the children ranged from 1 to 6 years, the median age was 3 years and 3 months. Inclusion criteria: children 1-6 years old with paralytic syndromes according to ICD-10 (cerebral palsy G80, hemiplegia G81, paraplegia and tetraplegia G82, other paralytic syndromes G83) on the background of CNS damage caused by hypoxia, bleeding, thrombosis, trauma; congenital brain defects. Exclusion criteria: malignant neoplasms; HIV/AIDS; degenerative diseases of the nervous system, demyelinating diseases; chromosomal diseases; orphan diseases; and those who did not consent to participate in the study.

DATA COLLECTION

The research included a detailed evaluation of medical records, including an "Individual Rehabilitation Plan". The Gross Motor Function Classification System (GMFCS) was used to provide a standardized assessment of limitations in gross motor function in patients with paralytic syndromes [3, 4, 6].

The rating scale r-FLACC (Revised - Face, Legs, Activity, Cry, Consolability Scale) was used in children with impaired communication skills, nonverbal children in order to perform the pain measurement. It is freely available on the Internet and is not marked with permission to its use [13]. The scale is based on determining the degree of pain by the sum of points: 0 points - relaxed/comfortable; 1-3 points - mild discomfort; 4-6 points - moderate pain; 7-10 points - severe pain. Pain evaluation on the r-FLACC scale was performed by parents together with a physical therapist.

The parents' interview was conducted using the "Original Questionnaire for Mothers/Legal Representatives (Guardians)" (Copyright №88107, May 2019). It includes five domains: Domain 1 – "Condition of child" (mobility, walking; pain or discomfort; child's ability to calm down; scream baby while feeling pain or discomfort); Domain 2 – "Score of Rehabilitation" (evaluation of the child condition before and after rehabilitation on a scale from 0 to 10, and number of rehabilitation courses, and follow rehabilitation recommendations at home); Domain 3 – "Parenteral emotional state" (anxiety, agitation, sleep and support - 13 items). Each item provided at least three choices: "Most of the time", "Sometimes" or "Almost never". This publication contains the results of the analysis of Domain 3 depending on Domain 1 (item Pain or discomfort).

Since 2020, when the "orange" and "red" quarantine zones for the COVID-19 pandemic were introduced, our institution has implemented online counseling using various platforms at the parents' request (Zoom, Google Meet, Viber, Telegram, FaceTime), as well as distribution of the original questionnaire for parents via e-mail (Google). Physical therapists conducted and complected the "Individual Rehabilitation Plan" together with parents. The physical therapist performed the distance learning and supervision of classes performed by parents at home. At their request, and if the epidemic situation allowed, physical therapists made homebased visits in accordance with the rules of hygiene. Physical therapists were interviewed periodically.

ANALYSIS

Statistical analysis was performed with the programs Statistica 7.0 StatSoft Inc.1984–2004, (Serial Number 12255555555, USA) and MedCalc version 14.8-© 1993-2014 MedCalc Software bvba (Acacialaan 22 B-8400 Ostend, Belgium). Descriptive analysis, comparison of two proportions were used. In-group changes were estimated with the help of frequency tables and crosstabulations function to have an opportunity to connect the frequency

Table I. Demographic and clinical data of the cohort of children with paralytic syndromes

Sign	Before the COVID-19 pandemic n=64 Abs. (%)	During the COVID-19 pandemic n=32 Abs. (%)
Age, Me*	3 years 4 months.	3 years 1 month.
Number of children less than 1 year old	7 (10.9)	3 (9.3)
Number of children between 1 and 3years old	25 (39.0)	14 (43.7)
Number of children 3 years old or above	32 (50.0)	15 (46.8)
Sex, males.	40 (62.5)	21 (65.6)
Sex, females.	24 (37.5)	11 (34.3)
Congenital pathology of the CNS	32 (50.0)	13 (40.6)
Cerebral palsy	21 (32.8)	10 (31.3)
Other factors of paralytic syndromes	11 (17.1)	9 (28.1)
Spastic tetraparesis	41 (64.0)	16 (50.0)
Spastic paraparesis	8 (12.5)	10 (31.3)
Paraplegia	11 (17.1)	6 (18.7)
Diplegia	4 (6.3)	-
GMFCS I - II	13 (20.3)	-
GMFCS III	9 (14.0)	6 (18.7)
GMFCS IV	12 (18.8)	13 (40.6)
GMFCS V	30 (46.8)	13 (40.6)
Anticonvulsant therapy	26 (40.6)	11 (34.3)

*Note: Me - median

Table II. Characteristics of chronic pain in a cohort of children with paralytic syndromes using the r-FLACC scale

Score	Before the rehabilitation Abs. (%)	After the rehabilitation Abs. (%)	p
Before the pandemic (2018-2019), n=64			
0-3 points	33 (51.5)	53 (82.8)	0.0003
4-6 points	18 (28.1)	8 (12.5)	0.0376
>7 points	13 (20.3)	3 (4.6)	0.0115
During the pandemic (2020), n=32			
0-3 points	22 (68.7)	24 (75.0)	0.5949
4-6 points	4 (12.5)	2 (6.25)	0.3433
>7 points	6 (18.7)	6 (18.7)	1.000

of display of observations at different levels. Logistic regression analysis using relative risk index (RR) and its 95% confidence interval (CI). The difference in parameters was considered statistically significant at $p<0.05$ by Fisher test and associated P-value with z-statistic.

RESULTS

A thorough study of the life history, assessment of clinical syndromes and movement disorders in all children at the beginning of the study were performed. Demographic and cumulative clinical data are given in table I.

The distribution of children according to demographic data, basic syndromes and movement disorders was homogeneous, which was due to the contingent of children who are provided with rehabilitation services in the institution. We note that there were more boys with paralytic syndromes (2018-2019 p=0.0076 and 2020 p=0.0129). Among the leading paralytic syndromes, the most common was spastic tetraparesis (2018-2019 p=0.0076 and 2020 p=0.0129). The frequency of children with level III – V motor disorders prevailed: 51 (79.6%) children in the period 2018-2019 and 100% of children in the period 2020 (2018-2019 p=0.0076 and 2020 p=0.0129). Thus, there was a tendency for parents of children with level I-II movement disorders during the COVID-19 pandemic not to use physical therapy services. This cohort of children was characterized by the fact that 37 (38.5%) children received anticonvulsant therapy.

Table III. Characteristics of chronic pain in a cohort of children with paralytic syndromes according to the subjective assessment of mothers

	Before the rehabilitation Abs. (%)	After the rehabilitation Abs. (%)	p
Before the pandemic (2018-2019), n=64			
Your child does not feel pain or discomfort	8 (12.5)	27 (42.2)	0.0001
Your child feels moderate pain or discomfort	38 (59.4)	22 (34.4)	0.0053
Your child feels severe pain or discomfort	15 (23.4)	12 (18.7)	0.4848
During the pandemic (2020), n=32			
Your child does not feel pain or discomfort	12 (37.5)	13 (40.6)	0.8069
Your child feels moderate pain or discomfort	10 (31.3)	9 (28.1)	0.7933
Your child feels severe pain or discomfort	10 (31.3)	10 (31.3)	1.000

The results of pain assessment of children with mild discomfort (0-3 points) using the r-FLACC scale in a 6-month time interval did not differ significantly before and during the pandemic - 51.5% and 68.7% (p=0.1385) (Table II).

Children who showed severe pain (more than 7 points) were prescribed pharmacological therapy: in the period 2018-2019, 13 (20.3%) children received medication for pain correction, similarly to 6 (18.7%) children involved in the study in 2020 during quarantine measures (nonsteroidal anti-inflammatory drugs, myorelaxants, gabapentin). Among the general cohort of young children with paralytic syndromes who visited our rehabilitation department - 19 (19.8%) had severe pain according to the r-FLACC scale, which required medical correction. All children who showed severe pain were more than 4 years old, had spastic tetraparesis and GMFCS IV-V movement disorders. Severe and moderate pain was registered in 30 (46.8%) children at the beginning of the study in the pre-pandemic period and in 10 (31.2%) children during quarantine measures (RR=1.5; 95% SI 0.87-2.75; p=0.1359).

The number of children with severe pain decreased to 9 (9.3%) from the general cohort after a 6-month interval. But there were no changes in the prevalence of children with severe pain occurred during the pandemic, although the percentage was insignificant. We assume that they need more time than 6 months to relieve pain. In general, severe, and moderate pain in the end-point study was demonstrated by 11 (17.1%) children before the pandemic and 8 (25.0%) children during the pandemic (RR=0.68; 95% SI 0.30-1.53; p=0.3621). Therefore, a such factor as the effect of quarantine measures on reducing pain in children during physical therapy for 6 months can be ruled out.

We noticed that after a 6-month interval of rehabilitation measures, the prevalence of children who had moderate pain and did not receive pharmacological treatment of pain significantly decreased in the period before the pandemic (RR=2.3; 95% SI 1.1-4.7; p=0.0358) and haven't changed during pandemic (RR=2.0; 95% SI 0.4–10.1; p=0.4032).

We found that pain assessment on the r-FLACC scale and subjective assessment of maternal pain (Domain 2 of the questionnaire "chronic pain") were different (Table III).

According to the subjective assessment of chronic pain (moderate and severe) by parents, its frequency was higher than in the objective study using the r-FLACC scale (Table 2), as in the beginning of the study (RR=1.8; 95% SI 1.4–2.3; p<0.0001) and at the end-point after 6 months (RR=3.2; 95% SI 2.1–4.9; p<0.0001). At the beginning of the study, 53 (82.8%) children had moderate and severe pain according to their mother's opinion, and after 6 months of rehabilitation measures - 44 (68.7%) in 2018-2019; 20 (62.5%) at the beginning of the study and 19 (59.3%) children at the end-point after 6 months of rehabilitation measures in 2020. Thus, a factor such as subjective assessment of maternal pain in children with paralytic syndromes increases the incidence of pain compared to objective assessment.

Because the focus of this study is chronic pain and the hypothesis of the study was that physical therapy is effective in chronic pain relieving in children with paralytic syndromes and positively affects the psychosocial status of mothers of children with special needs, Domain 3 – "Parenteral emotional state" was analyzed in parents, who noted the presence of moderate and severe pain in children, for the analysis of which the cross-stabilization function was used (n=51).

During the period 2018-2019 before the COVID-19 pandemic, after a 6-month rehabilitation course, the "fall asleep due to child health" decreased statistically significantly from 48/51 to 25/51 (RR=1.9; 95% SI 1.4–2.6; p<0.0001), "sleepless nights due to child's condition" from 44/51 to 23/51 (RR=1.9; 95% SI 1.3–2.6; p<0.0001), "intolerable observation of the child's condition" from 46/51 to 20/51 (RR=2.3; 95% SI 1.6–3.2; p<0.0001), "feelings of inner anxiety" from 44/51 to 21/51 (RR=1.9; 95% SI 1.4–2.5; p<0.0001). A significant proportion of these emotional states' parents considered present as "Sometimes". "Most of the time" was characterized by such feelings as "…it is unbearable to watch a child suffering", "… feeling of helpless or scared when a child was in pain". Such emotional states and needs of parents as "needs talked to anyone who could specifically help", "looking for sympathy or understanding from someone", "concern that a child is receiving pharmacological or non-pharmacological treatment for pain in the last few days", "concern child's ability to lead a normal life in the last few days", "worrying that you have taken too much care of your baby in the last few days", "feeling upset and angry when a child is constantly screaming when in

pain or unable to calm down when in pain" were rated as "Almost never".

During the period of 2020 during the COVID-19 pandemic after a 6-month course of rehabilitation under remote conditions of service provision, the parents' feeling of "intolerable observation of the child's condition" decreased statistically significantly from 18/20 to 10/20 (RR=1.8; 95% SI 1.1–2.8; p=0.0126), "sleepless nights due to the child's condition" from 15/20 to 8/20 (RR=1.8; 95% SI 1.0–3.3; p=0.0379). Other emotional states remained after physical therapy, but "Most of the time" parents began to note the need to seek compassion and understanding "needs talked to anyone who could specifically help", "feelings of inner anxiety", "a sense of possible trouble".

DISCUSSION

Most rehabilitation programs for children with paralytic syndromes for several decades have been aimed for normalizing motor functions, ensuring normal posture and independent functional activity of the child, regulating muscle tone, improving visual and auditory responses, supporting motor development and motor control, preventing joint contractures, etc. According to experts, setting individual realistic aims, setting priorities, informing the family, and strengthening its participation increase the effects of physical therapy [14].

But according to many researchers, the effectiveness of physical therapy in children with paralytic syndromes is not based on sufficient scientific evidence. The use of such indicators as the type of exercise, their frequency, intensity, and duration has been demonstrated to assess the effectiveness of physical therapy [15].

For example, a recent review of evidence-based approaches to physical therapy for children with cerebral palsy, published in 2019, demonstrates the fact that the effectiveness of most interventions is limited. Despite the recognition of the effectiveness of individual targeted approaches to rehabilitation, future research is needed to determine the best ways to improve functional outcomes in children [16].

Our own study did not include a thorough analysis of a particular approach, type of exercise and their intensity in the provision of rehabilitation services to children with paralytic syndromes. We used a multidisciplinary approach based on the individual needs of a young patient, using a time interval of 6 months, GMFCS. And the main task was to determine the impact of physical therapy on chronic pain in children with paralytic syndromes, objective and subjective assessment of their parents, and their emotional state. The study involved 96 children and their 96 caregivers (mostly mothers) during 2018-2020 and those who agreed to participate in the study. The research was conducted both in the pre-pandemic and in the pandemic periods with the introduction of "orange" and "red" quarantine measures in the country.

A review of publications from 1990 to 2011, which included studies that have identified parents' experiences with their child's therapy, where a quarter of the child's population was under five years old, children receiving physical and/or occupational therapy in a rehabilitation program, provided a conceptual basis for that the experience of parents is closely related to the quality of intervention for the child [17]. This review evaluated 13 studies (eight qualitative and five quantitative). Parents expressed different aspects of their experiences, had different needs, and needed time to establish a relationship with their child's physical therapist [18].

Our proposed questionnaire created the conditions for closer interaction between the physical therapist and parents because we considered the experience of the parents (their assessment for rehabilitation), their emotional state, the awareness that the child has chronic pain and the ultimate importance of the wider context of the child in the family.

Equally important is the fact that physical therapists have received feedback on children's subjective assessment of pain "through the eyes of parents" and their emotional state. One Finnish survey of 201 members of a multidisciplinary team and 311 physiotherapy service providers found that they tended to rate their family-oriented service positively. However, the study showed that the family-oriented approach increases with increasing experience of the specialist [19].

Therefore, our research and the proposed questionnaire can be used to identify areas for improvement not only by our team of specialists, but also for use in other rehabilitation practices.

We choose a 6-month interval to draw some conclusions about the effective components of physical therapy for children with paralytic syndromes. This decision was based on a study that examined a 6-month lifestyle intervention in children and adolescents with cerebral palsy. Researchers have shown with high confidence that lifestyle modifications during this period (physical therapy, counseling) increase social participation, reduce fatigue, reduce pain, improve the quality of life and mental health of children with paralytic syndromes, as demonstrated by our study [20].

Children with paralytic syndromes have a number of factors that cause pain [20]. There is little empirical evidence that pain is better assessed clinically nowadays. The search for pain assessment in recent decades has led to the development and use of standardized pain assessment tools. Moreover, research and extensive international cooperation in this area continues, especially in people with developmental delays and cognitive impairments [21].

The researchers recognize that pain in children with intellectual disabilities is a common and complex phenomenon. However, there are no standard educational components for caregivers or guardians of such children [22]. Our study focuses on information for parents and professionals that nonverbal children (including children from 1 to 6 years old) with paralytic syndromes may have chronic pain, and they should be monitored for pain. Parents with a physical therapist can use pain assessment tools and gain experience in measuring it.

We have chosen the r-FLACC scale among the existing tools for assessing pain in nonverbal children. The selection of this scale was based on the studies of clinicians from three medical centers who viewed 15 videos of observations of children with neurological disorders using three pain assessment tools and preferred this scale. In addition, clinicians' scores correlated with parental scores ($p<0.001$) and the reliability of testing and retesting was confirmed by strong correlations ($r=0.8$-0.883; $p<0.001$) [23].

In our opinion, the interesting study were performed from 2011 to 2014. The definition of pain in a cohort of children with developmental disabilities (n=544) who attended an outpatient clinic was studied. The average age of children was 14 years in contrast to our research. A third of all children were suffered from cerebral palsy. Children were diagnosed with emotional disorders (anxiety and depression) accompanied by chronic pain, and even one that required treatment at the tertiary level of care. The authors consider that pain assessment should be a routine practice for all multidisciplinary teams [24].

The focus of our study was to examine the effect of a 6-month course of physical therapy on chronic pain in children with paralytic syndromes. We obtained data on the reduction of the frequency of moderate pain in children after 6 months without pharmacological intervention almost twice (RR=2.3; 95% SI 1.1–4.7; $p=0.0358$) in the pre-pandemic period. This confirms the fact that physical therapy is an integrative method of treating chronic pain in young children, which helps to optimize their health.

The evaluation and re-evaluation of chronic pain in children by their parents is still a debatable issue. Our study of subjective assessment of parents for moderate and severe pain in children with paralytic syndromes according to the questionnaire and comparing its frequency with objective assessment using the r-FLACC scale showed that parents overestimate the frequency of pain in children. The obtained results coincide with the data of the study of the reliability of parental pain assessments in children with cognitive impairment under the same methodology (using the FLACC scale simultaneously with nurses) [25]. This is due to the emotional and psychological state of the parents of children with neurological disorders.

According to one study, the psychological and physical health of caregivers of children with paralytic syndromes was highly dependent on the child's behavior and childcare requirements. Problems with children's behavior were an important factor in the psychological well-being of guardians, both directly and indirectly, because of their impact on self-perception and family functions [26]. These data confirm the influence of the emotional state of mothers directly in the first place on the emotional state of children and vice versa [27].

Strategies of optimizing of the caregivers' physical and psychological health include support for behavioral management and daily functional activity, as well as stress management techniques. Therefore, it is very important for specialists of multidisciplinary teams to know the emotional state of parents to correct their quality of life, the effectiveness of clinical interventions and care [28].

We identified some emotional problems in this study, and we hypothesized, that physical therapy would help to improve parents' emotional state. Thus, in the pre-pandemic period, a 6-month rehabilitation course allowed us to improve falling asleep (RR=1.9; 95% SI 1.4–2.6; $p<0.0001$) and sleep (RR=1.9; 95% SI 1.3–2.6; $p<0.0001$), reduce anxiety about the child's health (RR=2.3; 95% SI 1.6–3.2; $p<0.0001$) and reduce feelings of internal anxiety (RR=1.9; 95% SI 1.4–2.5; $p<0.0001$). Although we did not get a reduction in most other emotional disorders.

Another subgoal of our study was to identify changes in the impact of physical therapy on chronic pain in children with paralytic syndromes and the emotional state of their parents during the COVID-19 pandemic. Rehabilitation services were provided remotely as counseling by means of telecommunications and full involvement of parents in the implementation of exercises during of quarantine. With the advent of the SARS-CoV-2 virus pandemic, critical aspects of the management and care of these children arose, because they did not have access to the facility's services [29].

Now, it is essential to accumulate scientific knowledge and a larger contingent of children and their families, as they are in a crisis. Psychotherapeutic interventions and social support for such families should be strengthened.

LIMITATIONS

There were some inherent limitations associated with this study; firstly, sample size. Our model was based on non-invasive single-center cross-sectional study and was limited by the time and number of children with paralytic syndromes ant their parents. Secondly, there were very few prior research and gaps in the same studies. Our study was limited by the age of children from 1 till 6 years old.

There were confounders such as convulsions, pharmacological treatment of severe chronic pain. Third, this study did not examine the evaluation of service users, so it cannot be commented on. Fourth, the psycho-emotional state was studied mostly in mothers without determining their educational, family, and social status, and the results obtained are not quantifiable. Fifth, the attitude of all rehabilitation stakeholders to providing services during the COVID-19 pandemic has not been proven, so some remarks have been made. The results of statistical processing of the proposed questionnaire are not given in full, and the reliability of this tool should be evaluated in future reviews.

CONCLUSIONS

The authors consider that physical rehabilitation in children with paralytic syndromes reduces the incidence of moderate chronic pain and improves the emotional state of parents. But, these changes do not occur during the pandemic. So, we think that new approaches to rehabilitation should be applied not only with the help of telecommunications, but with daily management of the children with paralytic syndromes' needs and their families with

increased psychological and social support. Perhaps the searching for new approaches that optimize more intensive and effective rehabilitation strategies using the family reserve will provide the potential for adaptability of nerve plasticity and recovery in such a contingent of children and prospects for the future.

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ORIGINAL ARTICLE

THE VIABILITY OF LEUKOCYTES AND REACTIVE OXYGEN SPECIES GENERATION BY THEM IN RATS WITH CHRONIC COLITIS

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ABSTRACT

The aim: To assess reactive oxygen species production by leukocytes and their viability in rats with chronic colitis.

Materials and methods: Reactive oxygen species production was estimated in leukocytes, isolated from rats with Dextran Sulfate Sodium-induced chronic colitis and control rats, by flow cytometry using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate. Leukocyte viability and apoptosis stages were assessed by flow cytometry using annexin V and 7-aminoactinomycin D staining. White blood cell counting was carried out with using Hematology Analyzer.

Results: The increased fluorescence intensity of 2',7'-dichlorofluorescein in viable leukocytes by 36.7% was revealed in rats with chronic colitis compared control rats. A significant decrease in the percentage of viable cells and an increase in apoptotic cells were found compared to intact animals. Leukocytes, granulocytes, monocytes, lymphocytes counts in blood of experimental group animals were significantly higher compared to control those.

Conclusions: Our findings indicate that Dextran Sulfate Sodium-induced chronic colitis increases an intracellular production of reactive oxygen species by leukocytes. Despite of increased leukopoiesis it reduces viability of white blood cells and promotes their apoptosis via stimulation of oxidative stress.

KEY WORDS: leukocytes, rats, reactive oxygen species, apoptosis, colitis

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are two major forms of inflammatory bowel diseases (IBD). Oxidative stress is considered to be one of the main mechanisms involved in the pathophysiology of IBD [1, 2]. However, it should be noted that most studies that examined the interrelation between IBD severity and oxidative stress were not able to show a significant correlation between them [3].

Ulcerative colitis is also associated with excessive neutrophil infiltration [4]. In inflammation the main source of reactive oxygen species (ROS) in tissue are neutrophils and monocytes. Reactive oxygen species formed by neutrophils are necessary not only to eliminate the pathogen, but also for neutrophil apoptosis [5]. Neutrophil apoptosis plays an important role in an inflammation resolution [6]. Delayed neutrophil apoptosis leads to chronic inflammatory diseases [7]. Although ROS directly promotes and/or regulates neutrophil apoptosis, there is no consensus on the role of ROS on their lifespan [6].

Data on the relationship between ROS levels and severity of IBD are contradictory. Thus, according to Delson L A et al. (2018), mutations in genes encoding NADPH oxidases in children with CD are associated with reduced ROS production and a more aggressive course of the disease [8]. On the contrary, the reduced colonic inflammation during DSS-induced colitis and less ROS generation by bone marrow neutrophils after lipopolysaccharide stimulation are observed in dendritic cell immunoreceptor 1-knockout mice compared to wild type [9]. In addition, the blood plasma of patients with UC has attenuated inhibitory effects on the formation of ROS compared with the blood plasma of healthy people [10].

The study of ROS generation by blood leukocytes, their counts and apoptosis in DSS-induced colitis rats will not only clarify the relationship between ROS production and apoptosis, but also assess the possibilities of environmental influences (blood plasma) on these processes and an involvement the changes in these processes in leukocytes to extragastrointestinal manifestations of colitis.

THE AIM

The aim is to assess reactive oxygen species production by leukocytes and their viability in rats with chronic colitis.

MATERIALS AND METHODS

The study used 12 adult WAG rats, divided into two equal groups. Control group rats were fed on a standard diet. Water was provided ad libitum. Experimental group included the rats with chronic colitis induced by oral administration of 2.5% solution (weight/volume) Dextran Sulfate Sodium (DSS) (molecular weight: 40 kDa; PanReac AppliChem, Germany) in drinking water according the scheme: from

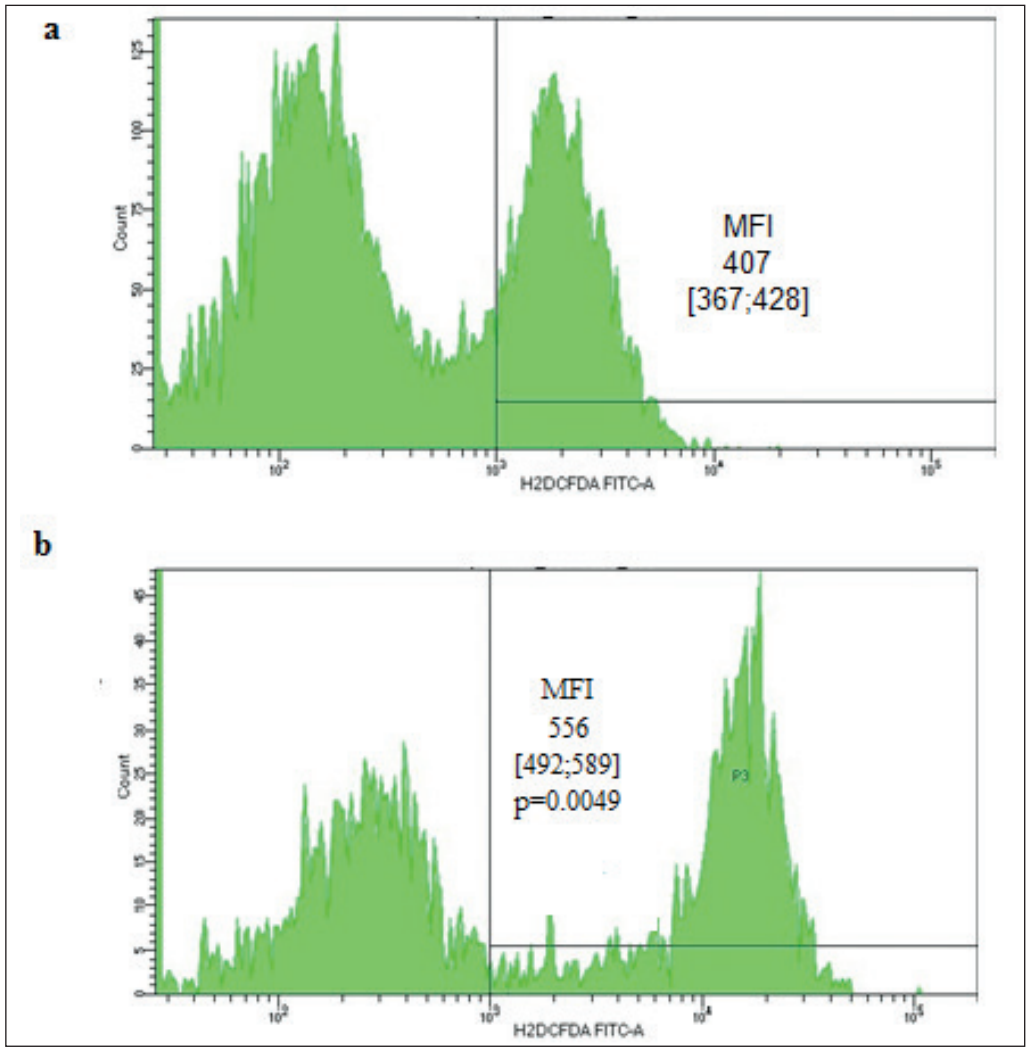

Fig. 1. Representative SSC/FL1 (2',7'-dichlorofluorescein diacetate, H2DCFDA) histograms of a control animal (a) and a rat with DSS-induced chronic colitis (b). Mean fluorescence intensity (MFI) of 2',7'-dichlorofluorescein (DCF) in CD45+, 7-AAD– cells is represented as Me [25%; 75%].

1st to 5th days, from 13th to 17th days, from 25th to 29th days the animals from the experimental group were orally administered DSS solution in drinking water; from 6th to 12th days, from 18th to 24th, from 30th to 38th days they received drinking water. On day 39, the animals were removed from the experiment with a guillotine knife [11-12].

Blood samples were collected to sterile vacutainer test tubes (IMPROVACUTER Evacuated EDTA K2 Spray Dried PET Tubes, Guangzhou, China) containing ethylenediaminetetraacetic acid dipotassium salt (K2EDTA). Blood samples were used for clinical blood test and leukocyte suspension preparation. To prepare leukocyte suspensions, aliquots of blood (100 µl) obtained from each animal were lysed and washed twice with Pharmlyse solution (Becton Dickinson, San Jose, USA) and phosphate-buffered saline (PBS), respectively. Leukocyte suspensions were used for further evaluation of ROS levels in living leukocytes and for analysis of leukocyte viability and their death modes.

To detect intracellular ROS concentration, the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) was employed. It is cleaved by intracellular esterases to form 2',7'-dichlorodihydrofluorescein, which is transformed by ROS into highly fluorescent 2',7'- dichlorofluorescein (DCF). Fluorescence degree of last reflects the intracellular ROS levels. According to the staining protocol used, in order for discrimination of leukocytes their suspensions in 100 µl PBS were stained with 10 µl APC-Cy™ 7 mouse anti-rat CD45 (BD Pharmingen, USA) for 15 minutes. The viable cells in the $CD45^+$ cells regions were then selected by staining with 5 µl 7-aminoactinomycin D (7-AAD, BD Pharmingen, USA) for 15 minutes. When cell membrane integrity is impaired, 7-AAD can enter the cell and bind to double-stranded DNA. The viable leukocytes were negatively stained with 7-AAD. So, $CD45^+/7AAD^-$ cells were incubated with freshly prepared 5 µM H2DCFDA (InvitrogenTM, USA) working solution in PBS at 37°C for 30min. The FL-1 channel was used to detect the fluorescence of DCF. The mean fluorescenceintensity (MFI) of DCF was analyzed using BD FACSDiva™software (Becton Dickinson, USA) for quantitative assessment of intracellular ROS production.

The viability of leukocytes and cell death modes were assessed with flow cytometry. Leukocyte suspensions (100 µL) were incubated for 15 min in the dark at room temperature with 10 µL APC-CyTM 7 mouse anti-rat CD45 (Becton Dickinson, USA), 5 µL FITC Annexin V, and 5 µL 7-AAD (Becton Dickinson, USA). Then 400 µL of 1 × binding buffer was added to each test tube. Flow cytome-

Table I. Mean fluorescence intensity (MFI) of 2',7'-dichlorofluorescein (DCF) in ROS intermediate/high CD45+, 7-AAD– cells of rats with DSS-induced chronic colitis

Groups of animals	Median	25% Percentile	75% Percentile
Control group	401.0	367.0	427.8
Experimental group	555.5 **	492.3	589.0

Note: ** - P <0.01 compared to the control group

Table II. Viability and cell death modes of leukocytes in rats with DSS-induced chronic colitis

Parameters	Control group			Experimental group		
	Median	25% Percentile	75% Percentile	Median	25% Percentile	75% Percentile
Viable leukocytes (%)	97.60	96.95	98.30	66.90 **	65.25	69.75
Early apoptotic cells (%)	0.65	0.40	0.83	25.40 **	24.00	26.93
Late apoptotic/ necrotic cells (%)	0.95	0.40	1.30	4.75 **	3.15	5.35
Dead necrotic leukocytes (%)	0.80	0.80	0.90	2.85 **	2.55	4.83

Note: ** - P <0.01 compared to the control group

try was performed with a FACSCanto™ II flow cytometry system (Becton Dickinson, USA) counting 10,000 events per measurement. FACSDiva™software (Becton Dickinson, USA) was used to assess the flow cytometry results. First of all, the region of $CD45^+$ cells was gated. Then, Annexin V and 7-AAD stained $CD45^+$ cells were analyzed. Dye-conjugated annexin V is able to label phosphatidylserine (PS), an early marker of apoptosis, on the extracellular membrane. In late-stage apoptosis, when cell integrity is damaged, it binds PS on the inner side of plasma membrane. 7-Aminoactinomycin D is fluorochrome which binds to double stranded DNA and can be used to stain non-viable cells. This staining is used to identify four possible states of leukocytes: 1, viable leukocytes (Annexin V^-, 7-AAD^- cells); 2, early apoptotic cells (Annexin V^+, 7-AAD^- cells); 3, late apoptotic/necrotic cells (Annexin V^+, 7-AAD^+); 4, dead necrotic cells (Annexin V^-, 7-AAD^+).

Lymphocyte, monocyte, granulocyte and total leukocyte counts were determined with using ABX Micros 60 ES Hematology Analyzer, France (10 µl of whole blood were used for analysis).

Statistical data processing was carried out using GraphPad Prism 5 Software (GraphPad Software, USA). Comparisons between two independent groups of variables were performed using a non-parametric Mann–Whitney U test. Results are represented as medians and interquartile ranges. Differences were considered significant at $p<0.05$.

The research was carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123). The Commission of Ethics and Bioethics approved the study design (Kharkiv National Medical University, Kharkiv, Ukraine; Protocol #6 d.d. 2 October 2019).

RESULTS

Analysis of DCF fluorescence in $CD45^+$, 7-AAD^- cells (viable leukocytes) revealed the fluorescence intensity increase in rats with chronic colitis by 36.7% compared control rats (fig. 1, table I). This indicates significantly elevated ROS levels in such subpopulations of WBC, as monocytes and granulocytes, in rats with DSS-induced chronic colitis compared to the control.

At the same time, the study of viability and apoptosis of leukocytes in the blood of experimental rats showed a significant decrease in the percentage of viable cells and an increase in apoptotic cells compared to intact animals (table 2). In the percentage the viable $CD45^+$ cells decreased by 1.5-fold ($p<0.01$), apoptotic cells increased (early apoptotic cells by 38.6-fold, $p<0.01$; late apoptotic/necrotic cells by 5-fold, $p<0.001$; dead necrotic leukocytes by 3.6-fold, $p<0.01$) in blood of experimental group rats compared to control group. This argues a greater predisposition to apoptosis and less stability of leukocytes in rats with chronic colitis compared with controls.

Nevertheless, the total number of leukocytes and counts of every of their fractions (granulocytes, monocytes, lymphocytes) in blood of experimental group animals were significantly enhanced compared to control those. Thus, the number of leukocytes increased by 62.6% (8.05×10^9 vs. 4.95×10^9, $p<0.01$), lymphocytes by 58% (5.85×10^9 vs. 3.7×10^9, $p<0.01$), granulocytes by 33% (1.4×10^9 vs. 1.05×10^9, $p< 0.01$), monocytes by 216.7% (0.95×10^9 vs. 0.3×10^9, $p<0.01$) in the blood of rats with colitis compared with control group rats.

DISCUSSION

Neutrophils are the most numerous fraction of phagocytic white blood cells; therefore, it is neutrophils that are the main

source of ROS in our study. Neutrophil-produced ROS are involved not only in the pathogen neutralization, but also in the neutrophil apoptosis regulation [5, 13]. ROS generation is triggered by NADPH oxidase. The induction of NADPH oxidase assembly in the plasma membrane leads to release of superoxide to the extracellular compartment or into preformed phagosome. Contrary, the induction of of NADPH oxidase assembly in the intracellular membrane (probably the granule membrane) results in intracellular production of superoxide [14]. According to the review work of Karsten Kruger, the sustained excessive ROS production can result in apoptotic cell death by the intrinsic pathway of apoptosis [15]. In our study namely intracellular ROS were determined. We found elevated levels of ROS in the blood leukocytes of the experimental group animals compared with the controls. And the increased ROS generation was accompanied by enhanced apoptosis and reduced viability of leukocytes.

We speculate that the intensive production of ROS by leukocytes of these animals is due to their activation in the bloodstream under the influence of pro-inflammatory cytokines and/or the programed altering expression of related to apoptosis proteins (death receptors, proapoptotic or antiapoptotic proteins) on differentiaton and maturation stages. This assumption is confirmed by literature data. Thus, according to study of Avdagić N et al. [16], TNF elevated levels were found in patients with CD and patients with UC as compared healthy persons. According to the literature, the effect of TNF on the survival of neutrophils depends on its concentration: at low concentrations it increases the survival of neutrophils, at high - aggrevates their apoptosis [17]. The opposite TNF-α effects are caused by involving different elements of antiapoptotic machinery: caspase-dependent turnover of the antiapoptotic protein Mcl-1 in high concentrations and TNF-α - mediated activation of Bfl-1 expression in low concentrations [18].

In addition to that, neutrophil subpopulations are distingueshed by their sensitivity to the same TNF-α concentration: one of them rapidly losses the mitochondrial membrane potential and is involved into apoptosis; the other one has elevated mitochondrial membrane potential that facilitates an apoptosis delay [18]. In a review article [13] it is noted that the reduction of transmembrane potential of mitochondria promotes the release of proapoptotic proteins such as: cytochrome c, second mitochondria-derived activator of caspases (Smac)/DIABLO (direct IAP-binding protein with low pl), apoptosis-inducing factor (AIF), and endonuclease G.

So, our data indicate increased myelopoiesis and lymphopoiesis in animals with chronic DSS-induced colitis. However, the ratio between the individual fractions of leukocytes is practically unchanged, with the exception of monocytes. The intensified ROS generation and the increased leukocyte apoptosis were observed in rats with chronic DSS-induced colitis.

CONCLUSIONS

Our findings indicate that DSS-induced chronic colitis increases an intracellular production of ROS by leukocytes. Despite of increased leukopoiesis it reduces viability of white blood cells and promotes their apoptosis via stimulation of oxidative stress.

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ORIGINAL ARTICLE

FEATURES OF THE COMORBID COURSE OF CHRONIC PANCREATITIS AND ARTERIAL HYPERTENSION

DOI: 10.36740/WLek202209217

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ABSTRACT

The aim: To investigate the clinical features of the chronic pancreatitis (CP) clinical course in patients with concomitant arterial hypertension.

Materials and methods: 100 patients with PD were investigated. In 60 patients, the course of CP and AH was combined - the main group, the comparison group - 40 patients with CP without concomitant pathology.

Results: In 52 patients (86.7%) with CP and AH abdominal pain was recorded versus 24 (60.0%) with CP ($p<0.01$). Correlation analysis revealed weak relationship between the intensity of pain acc. Visual analogue scale (VAS) of pain and the degree of steatorrhea ($\tau = 0.40$, $p <0.01$), the degree of amilorrhea ($\tau = 0.39$, $p <0.01$) and the average strength of the relationship with creatorrhoea ($\tau = 0.60$, $p <0.01$). Dyspepsia was revealed in CP and AH: flatulence in 55 (91.7%) compared with 26 (65.0%) with CP, diarrhea in 52 (86.7%) patients in the main group versus 23 (57.5%) in the comparison group, nausea in 52 (86.7%), vomiting in 45 (75.0%) in the main group versus 18 (45.0%) and 12 (30.0%) patients from the comparison group ($p<0.01$ in all comparisons). Asthenia is expressed in patients with CP and AH: weakness in 50 (83.3%) patients versus 6 (15.0%), psychoemotional lability in 44 (73.3%) versus 3 (7.5%), headache in 47 (78.3%) versus 6 (15.0%), sleep disorders in 45 (75.0%) compared with 1 (2.5%) patients with CP ($p<0.01$ in all comparisons).

Conclusions: The negative effect of concomitant hypertension on the clinical course of CP has been established. AH contributes to increased pain syndrome, dyspepsia, asthenia.

KEY WORDS: diagnosis, chronic pancreatitis, arterial hypertension, course, clinical picture

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INTRODUCTION

The modern clinical practice has a number of features - the comorbidity of various chronic non-infectious diseases of internal organs, during which, given the possible intersection of pathogenetic links or their combination, a complication can form, which also affects the quality of life of patients. With the greatest frequency, such a combined course refers to the digestive tract diseases, which are recorded in almost more than 60% of the population and diseases of the cardiovascular system, which, in terms of their prevalence, disability and mortality rates, rank first in the morbidity patterns [1,2]. According to world statistics, CP in the morbidity patterns of the digestive system ranges from 5.1 to 9%, and in the structure of general clinical practice ranges from 0.2 to 0.6%. According to research in Ukraine, the incidence rate of pancreatic pathology in 2016 amounted to 226 cases per 100 thousand of population, prevalence - 2471 per 100 thousand of population [3]. Hypertension is the most common disease in the world. The global prevalence of hypertension is 31.1% according to the results of the systematic analysis of population studies from 90 countries [4]. Being, on the one hand, the independent disease, and on the other hand, a generally recognized risk factor for chronic non-infectious diseases, hypertension is of interest for a comprehensive study of the comorbidity of this pathology. The high prevalence of the gastrointestinal tract diseases and hypertension in the general population determines the relevance of the search for possible associations between them. Despite significant progress in understanding the mechanisms of CP and Hypertension and improving approaches to their treatment, pancreatitis and hypertension remain the most influential group of the diseases that significantly impair the quality of life of patients and lead to death, especially in comorbid patients [5,6]. Thus, the study of the clinical and laboratory features of the comorbid course of CP and AH is the urgent problem of internal medicine and the important aspect of further dynamic monitoring.

THE AIM

The aim of this work was to study the clinical features of the chronic pancreatitis clinical course in patients with concomitant arterial hypertension.

MATERIALS AND METHODS

To assess the clinical course of combined and isolated CP, we have carried out a thorough analysis of the disease history, the results of clinical and laboratory examination of 100 patients with chronic pancreatitis who were on ambulatory care in the Outpatient department of the

Table I. Risk factors for the CP development in the examined patients

Risk factors	All patients (n = 100), abs. (%)	Patients with CP and AH (n = 60), abs. (%)	Patients with CP (n = 40), abs. (%)	p
Nutritional	75 (75.0%)	37 (61.7%)	38 (95.0%)	<0.01
Biliary	40 (40.0%)	23 (38.3%)	17 (42.5%)	0.68
Alcoholic	34 (34.0%)	15 (25.0%)	19 (47.5%)	0.02
Smoking	54 (54.0%)	34 (56.7%)	20 (50.0%)	0.51
Gastro-duodenal	28 (28.0%)	19 (31.7%)	9 (22.5%)	0.32
Obesity	54 (54.0%)	35 (58.3%)	19 (47.5%)	0.29
Psycho-emotional	50 (50.0%)	37 (61.7%)	13 (32.5%)	<0.01

Table II. Pain intensity acc. VAS in the examined patients

Parameter	All patients (n = 100)	Patients with CP and AH (n = 60)	Patients with CP, (n = 40)	p
Pain intensity acc. VAS: 2 scores , n (%)	18 (18.0)	0 (0.0)	18 (45.0)	0.008
Pain intensity acc. VAS: 3 scores , n (%)	25 (25.0)	4 (6.7)	21 (52.5)	0.0013
Pain intensity acc. VAS: 4 scores , n (%)	19 (19.0)	18 (30.0)	1 (2.5)	0.0098
Pain intensity acc. VAS: 5 scores , n (%)	20 (20.0)	20 (33.3)	0 (0.0)	0.0047
Pain intensity acc. VAS: 6 scores , n (%)	18 (18.0)	18 (30.0)	0 (0.0)	0.0019

Table III. Blood pressure level and heart rate in the examined patients

Parameter	All patients (n = 100)	Patients with CP and AH	Patients with CP,	p
Systolic blood pressure, mm Hg	150.0[120.0;155.0]	155.0[150.0;160.0]	120.0 [120.0;130.0]	<0.01
Diastolic blood pressure, mm Hg	95.0 [80.0;96.2]	95.0 [95.0;100.0]	80.0 [70.0;80.0]	<0.01
Heart rate, beats / min.	84.0 [78.0;94.5]	92.0 [85.5;98.0]	76.0 [72.0;80.0]	<0.01

Dnipropetrovsk Regional Clinical Hospital named after I. I.I. Mechnikov in 2019-2020. Written voluntary informed consent to participate in the study was obtained from each patient. Among 100 patients with CP, 60 were associated with CP and AH, they made up the main group and the comparison group - 40 CP patients without concomitant pathology of the cardiovascular system. The average age of the examined patients was 51.5 [49.1; 59.1] years. In the comparison group, the distribution of patients by age and sex was compared with the main group.

CP was diagnosed on the basis of anamnesis data, clinical manifestations and results of laboratory and instrumental studies, taking into account the recommendations of the Joint European Gastroenterological Association for the diagnosis and treatment of chronic pancreatitis, based on evidence (2017). The blood pressure assessment was based on the basic requirements of the "Unified clinical protocol for primary, emergency and secondary (specialized) medical care for arterial hypertension", approved by the Order of the Ministry of Health of Ukraine No. 384 dated May 24, 2012, Clinical guidelines for arterial hypertension of the European Society of Hypertension and the European Cardiological society (2013 and 2018).

Determination of indicators of the general blood test, biochemical blood test, general urine analysis and coprograms were carried out according to the generally accepted methods to estimate general characteristics of patients. The electrocardiogram was recorded to determinate heart rhythm disorders, the ultrasound examination of the abdominal organs was performed according to the generally accepted rules with accessing state of pancreas. All subjects underwent anthropometric studies to determine obesity by BMI according to the Quetelet formula: BMI = weight (kg) / height (m2). Pain intensity was assessed using a 10-point visual analogue pain scale (J. J. Bonica, 1990). Data processing and statistical analysis were performed using the LibreOffice and R software packages (version 3.6.3) (1) [7]. More than 50% of the data had a different than normal type of distribution according to the Shapiro-Wilk test, so the analysis used non-parametric statistics, the data were described as the median and 25 and 75 quartiles (Me [25%; 75%]. Comparing quantitative indicators, the Mann – Whitney test was used; Pearson's Chi-square test ($\chi 2$) was used to compare qualitative indicators. Correlation analysis was performed using the non-parametric Spearman correlation coefficient (ρ). The trend lines on the charts correspond to the linear regression lines. The significant level of p for statistical hypotheses is taken <0.05

RESULTS

There were no statistically significant differences in gender distribution between the groups, but in the group of patients with CP and AH, women predominated 63.3%,

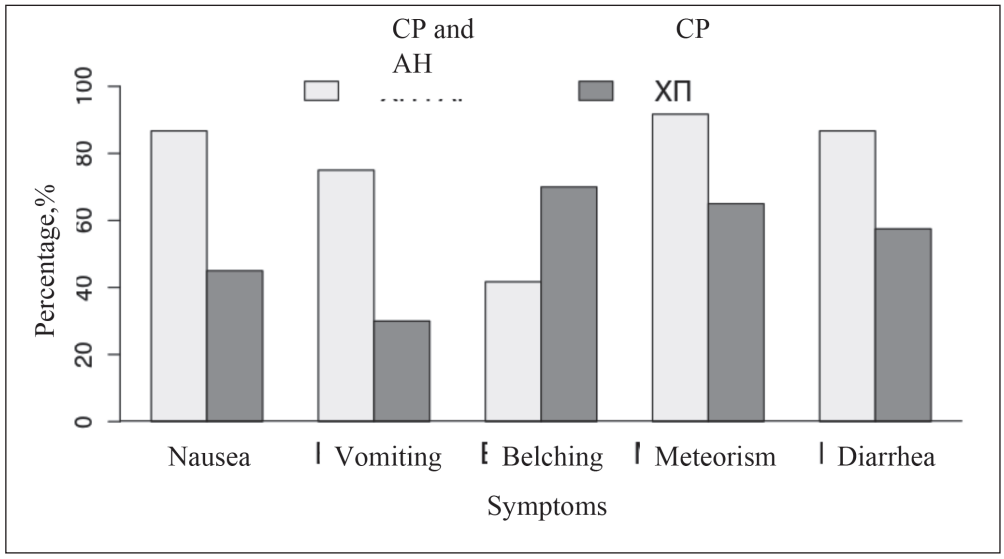

Fig. 1. Frequency of dyspepsia symptoms in patients with CP and AH and CP

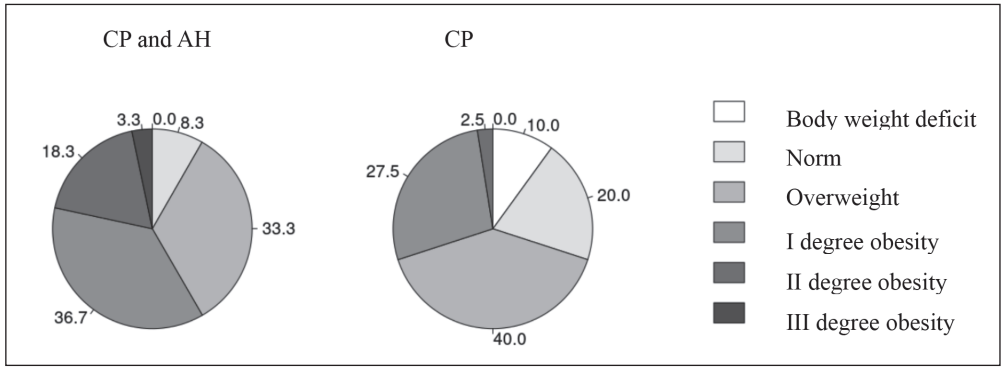

Fig. 2. Structure of BMI categories of patients with CP and AH and CP

and in the group with CP, the ratio of men and women was almost the equal. The median age in the main group was significantly higher by 6.5 years than in the comparison group ($p = 0.01$), the average age in the group of patients with CP and AH was 57.0 [47.0; 62.0] years, in the comparison group 50.5 [50.0; 52.1] years. The duration of CP disease ranged from 4 to 9 years; AH was followed by anamnestics from 3 to 10 years. Of these diseases, 55% of patients were preceded by CP, and 45% by AH.

The anamnestic data of patients with CP were analyzed to identify risk factors in the main group and the comparison group (Table I). With the combined course of CP and AH, the psychoemotional factor was more often observed in 37 (61.7%) patients compared with 13 (32.5%) patients in the CP group ($p<0.01$). In the presence of the isolated course of CP, the nutritional risk factor prevailed in 38 (95.0%) versus 37 (61.7%) patients with the combined course of CP and AH ($p<0.01$). The results obtained correspond to the data of scientists studying this problem [2,8-10].

Studying the clinical symptoms features it should be noted that with the combined course of HP and HA in patients with the main group the pain syndrome was recorded in the pancreatic projection zone in 41 (68.3%) of the patient in comparison with 3 (7.5%) with HP ($p <0.01$) and more often its occurrence in the main group was noted against the background of the increase in blood pressure (BP). The pain after eating was more often observed with the isolated course of CP - in 39 (97.5%) patients compared with 23 (38.3%) patients with CP and AH ($P<0.01$), which caused the patients of the comparison group to limit the frequency and amount of food. The overwhelming majority of the main group patients determined pain as permanent discomfort in the abdominal cavity with the periodic amplification in 52 (86.7%) patients, at that time, in the comparison group, most observed the periodic character of pain - 24 (60.0%) of the patient ($P<0, 01$).

The Visual analogue scale medians were statistically significantly higher in the group with HP and AH (Table II).

The correlation analysis showed a weak relationship between the pain intensity according to Visual analogue scale and the degree of steatorrhea ($\tau = 0.40$, $p <0.01$), the degree of amylogory ($\tau = 0.39$, $p <0.01$) and the medium power relationship with the creatorrhoea ($\tau = 0.60$, $p <0.01$).

The presence of abdominal pain syndrome in the surveyed patients was associated with the decrease in the exocrine function of the pancreas. Reducing the generation of enzymes, decrease in the production of bicarbonate of the pancreas leads to excessive acidification of the initial departments of the duodenum, violation of motility and the natural development of such symptom as a meteorism and pain.

In our study, patients were sometimes very difficult to distinguish that they were concerned about: meteorism or pain. These symptoms were subjectively intersecting between each

other and influenced the quality of life of patients. The pain occurrence in the combined course of CP and AH, we also associated with the systemic damage to the vascular bed, which led to the activation of the inflammatory component in endothelial dysfunction and with metabolic components.

The frequency analysis of the dyspeptic complaints manifestations revealed (Fig. I) that most frequent symptom of dyspepsia in the examined patients was meteorism - 81 (81.0%). In the presence of the combined course of diseases in patients of the main group on the phenomenon of flatulence complained 55 (91.7%) in comparison with 26 (65.0%) patients with HP ($p<0.01$). Unformed chair, diarrhea was observed in 75 (75.0%) patients. The presence of diarrhea was disturbed 52 (86.7%) of patients of the main group against only half of patients 23 (57.5%) from the comparison group ($P<0.01$). Frequent manifestations of these dyspepsia symptoms in patients with the main group can be associated with large pancreas secretion oppression and disorders of the intestinal motility. Nausea and vomiting was most often recorded in patients of the main group: nausea in 52 (86.7%), vomiting in 45 (75.0%) against 18 (45.0%) and 12 (30%) of patients, respectively, from the comparison group ($P<0.01$) .

The dyspeptic syndrome in this sample of patients was due to the oppression of the pancreas secretion and violation of motility. When combining the course of CP and AH, such symptoms of dyspepsia as nausea and vomiting were also associated with the increase in blood pressure.

Overall weakness, sleep impairment, headache and psycho-emotional lability of varying degrees were more expressed in patients of the main group: total weakness of 50 (83.3%) against 6 (15.0%) patients, psycho-emotional lability in 44 (73.3%) compared with 3 (7.5%) patients, headache in 47 (78.3%) in comparison with 6 (15.0%), sleep disorders in 45 (75.0%) against 1 (2.5%) patient with CP ($P<0.01$ for all comparisons).

The presence of asthenovegetative syndrome in patients can be associated with the inflammatory component, metabolic disorders, as well as with impaired sleep and waking schedule.

When analyzing changes in the cardiovascular system, we recorded blood pressure increase and tachycardia in patients of the main group. Median of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) were statistically significantly higher in the CP and AH group (Table III).

After analyzing eating disorders in the compared groups, we found that the median BMI is 31.0 [27.0; 34.0] kg / m2 versus 28.0 [24.0; 30.0] kg / m2, Hip circumference (HC) - 106.0 [101.8; 110.0] cm compared to 98.5 [95.8; 104.2] cm, Waist circumference (WC) / Hip circumference (HC) ratio - 0.7 [0.6; 0.7] cm versus 0.7 [0.7; 0.7] cm, were statistically significantly higher in the group with CP and AH in comparison with patients with CP ($p <0.01$ in all comparisons). In the group with the presence of hypertension, the prevalence of I-II stages obesity (Fig. II) versus the group without AH 22 (36.7%) versus 11 (27.5%) and 11 (18.3%) versus 1 (2.5%), respectively ($p<0.01$).

It should also be noted that the level of fecal β-elastase was lower in patients of the main group - 111.5 [94.4; 146.2] μg of elastase per 1 g of feces compared with patients with chronic pancreatitis 153.6 [121.8; 181.2] μg of elastase per 1 g of feces ($p<0.01$).

DISCUSSIONS

Combined pathology is one of the main problems of modern medicine. The mononosological nature of the internal organs pathology study often becomes the cause of diagnostic errors, leads to a one-sided treatment nature, which in turn negatively affects the prevention of complications. The combined lesion of the digestive and cardiovascular systems is most often found in the clinic of internal diseases, due to the extreme prevalence of such diseases. Recent scientific studies have revealed a clear link between the course of CP and cardiovascular diseases, among which the leading place is taken by hypertension [2,7,9]. The blood pressure increase remains the main factor in the cardiovascular complications development, which in recent years has been the main cause of death in many countries of the world, including in our country. The combination of CP and AH is pathogenetically determined, primarily due to systemic damage to the vascular system, which leads to the endothelial dysfunction development, lipid metabolism disorders, activation of the inflammatory component, metabolic disorders [1,5,6]. Features of the clinical course of CP are often determined by its combination with hypertension, contributing to changes in the clinical picture of diseases. These changes in the course of diseases were observed in our study.

The study revealed that alcohol consumption and dietary disorders, as causes of CP, occurred in the group of patients with CP more often than in the group of patients with CP and AH ($p = 0.02$ and $p <0.01$, respectively). The psychosomatic cause of CP was more frequent in the group of patients with CP and AH compared with the CP group ($p <0.01$). Pain before meals was more often observed in patients with CP and AH ($p <0.01$), pain after eating was more often observed in patients with CP ($p <0.01$). The proportion of patients with persistent pain was significantly higher among patients with CP and AH ($p <0.01$) compared with the CP group ($p <0.01$). Patients with CP and AH more often rated pain at 4-6 scores on the VAS, while patients with CP more often rated pain at 2-3 points on the VAS (p for difference between groups <0.01). In the group of patients with CP and AH, nausea, vomiting, flatulence, diarrhea, general weakness, psychoemotional lability, headache, sleep disturbance and elastase level decrease in feces were significantly more frequent compared with the CP group (p for the difference between the groups <0.01) ... The median SBP, DBP, heart rate, OS, OT / V ratio, BMI and VAS were statistically significantly higher in the group of patients with CP and AH compared with the CP group (g for the difference between the groups <0.01).

CONCLUSIONS

Features of the clinical course of CP were determined by its combination with AH, which contributed to significant changes in the clinical picture of polypathia. The negative effect of concomitant AH on the clinical course of CP was established. AH contributes to increased pain syndrome, more pronounced manifestations of dyspepsia, asthenia, changes in nutritional parameters in patients with CP.

1. It was found that in the combined course of CP and AH, the onset of pain syndrome was noted before eating in 41 (68.3%) patients compared with 3 (7.5%) patients with CP ($p<0.01$). Pain after eating was more often observed in the

isolated course of CP - in 39 (97.5%) patients compared with 23 (38.3%) patients with CP and AH (p<0.01)
2. The VAS medians were statistically significantly higher in the group with CP and AH compared with CP (p <0.01). Correlation analysis showed a weak relationship between the intensity of pain according to the VAS and the degree of steatorrhea (τ = 0.40, p <0.01), the degree of amilorrhea (τ = 0.39, p <0.01) and the average strength of the relationship with creatorrhoea (τ = 0.60, p <0.01).
3. Dyspeptic syndrome was more often recorded in the combined course of CP and hypertension: 55 (91.7%) complained of flatulence in comparison with only 26 (65.0%) patients with CP, the presence of diarrhea concerned 52 (86.7%) patients of the main group against only half of patients 23 (57.5%) from the comparison group, nausea in 52 (86.7%), vomiting in 45 (75.0%) in the main group against 18 (45.0%) and 12 (30.0%) patients, respectively, from the comparison group <0.01 for all comparisons.
4. A characteristic feature of the combined course of CP and AH were manifestations of asthenovegetative syndrome: general weakness in 50 (83.3%) versus 6 (15.0%) patients in the comparison group, psychoemotional lability in 44 (73.3%) versus 3 (7.5%) patients with CP, headache in 47 (78.3%) versus 6 (15.0%), sleep disorders in 45 (75.0%) versus 1 (2.5%) CP patient (p<0.01 in all comparisons).
5. It was proved that the median BMI, HC, WC/ HC ratio were statistically significantly higher in the group with CP and AH compared with CP (p <0.01). In the group with the presence of hypertension, the prevalence of I-II stages obesity compared with the group without AH 22 (36.7%) versus 11 (27.5%) and 11 (18.3%) versus 1 (2.5%), respectively (p<0.01).

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ORIGINAL ARTICLE

MODELS OF PARASPINAL MUSCLE DEGENERATION IN RATS: HIGH-FAT DIET AND PROLONGED COMPRESSION

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ABSTRACT

The aim: To study the structural features of the lumbar m. multifidus and the m. psoas after keeping rats on a high-fat diet (obesity) or compressing their lumbar paraspinal muscles by binding the muscles using non-absorbable sutures.

Materials and methods: The study was performed on 2-month-old male rats (n=15) into three groups of 5: control group (normal diet without any surgical interventions), high-fat diet (model I: 40-45% kcal fat), and paraspinal muscles compression (model II: paraspinal muscles were tied from L2 to S1 with non-absorbable sutures Nurolon® 3). The experiment lasted for 90 days, after those fragments of the lumbar m. multifidus and m. psoas removed and histomorphometry analysis performed.

Results: 12 weeks from the beginning of the experiment, the high-fat diet rats weighed, on average, 22% (p=0.001) more than the control group rats. Similar degenerative changes such as uneven muscle fibre width and sarcoplasm colouring, 'wavy' and swollen fibres, loss of striation, karyopyknosis were observed in the lumbar paraspinal muscles in both models. In high-fat diet group the fat area (%) in the m. multifidus was 1.8 times larger (p<0.001) and in the m. psoas was greater by 2.2 times (p<0.001) than in the control. Fibrous tissue replaced muscle fibres in m. multifidus in model II and was 12.66%.

Conclusions: The relevance of the models is proven: after 3 months, it is possible to obtain degenerative changes in the muscle tissue that are extremely similar to those observed in the muscles of patients with degenerative spine diseases.

KEY WORDS: skeletal muscle fibers, lumbosacral region, animal disease model, obesity, ischemia, histology

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INTRODUCTION

One in two people experience low back pain during their lives, with paraspinal muscles most probably playing a role [1]. Newly published clinical research confirms the appearance of disturbances in the m. multifidus from the direction of the intervertebral disc herniation [2, 3]. Specifically, a decrease in the area of the m. multifidus [2], in the size of type I and II fibres, an increase in fat tissue infiltration and quantity of type I fibres, and muscle atrophy were observed [3]. Additionally, patients with intervertebral disc herniation had m. multifidus with an unusually large percentage of fibro-adipogenic progenitors, which may be linked to the increased fat tissue area and fibrosis in the paraspinal muscles [4]. Increased fat infiltration with age was discovered even in persons without back pain, mostly centered in the lumbar m. multifidus and the erector spinae [5, 6], but also in patients with spondyloarthritis [7, 8], spondylolisthesis, lumbar spinal stenosis [9]. However, it is not known whether disruptions in the paraspinal muscles lead to degenerative disturbances in the intervertebral disc and facet joints. At the same time, it was shown in experiments that muscle injuries or myopathy are a factor in the development of spinal kyphosis [10] and degeneration of the intervertebral disc [11].

Based on this information, we decided to model (on rats) paraspinal muscle disturbances that are observed in patients with degenerative spine diseases. We hypothesized that, as a result of the application of the first model (high-fat diet for the rats – obesity), the amount of fat contained in the m. multifidus would increase. Today, obesity is viewed as a chronical mild inflammation, which is linked to an increase in the level of circulating inflammatory cytokines — C-reactive protein, interleukins (IL), tumor necrosis factor α (TNFα) [12]. As a result of the impact of IL-1β, IL-10 and TNFα, degenerative disturbances can develop not only in paraspinal muscles, but also in the intervertebral discs [13]. For the second model, we chose a compression of the lumbar paraspinal muscles because mechanical compression leads to cell apoptosis and disruption of the structural integrity of the skeletal muscles [14].

THE AIM

The aim of this study was to study the structural features of the lumbar m. multifidus and the m. psoas after keeping rats on a high-fat diet (obesity) or compressing their lumbar paraspinal muscles by binding the muscles using non-absorbable sutures.

Fig. 1. The wound after the rat's lumbar paraspinal muscles were tied.

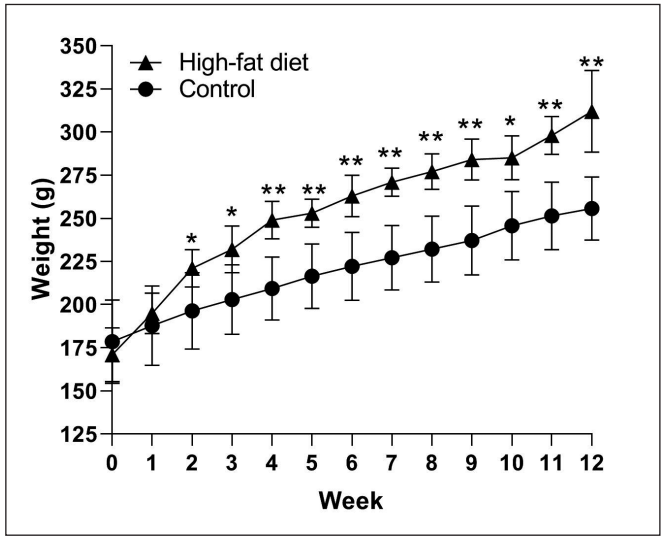

Fig. 2. Changes in the weight of rats during the experiment. Data is shown as mean±standard deviation. *p<0.05; **p<0.01.

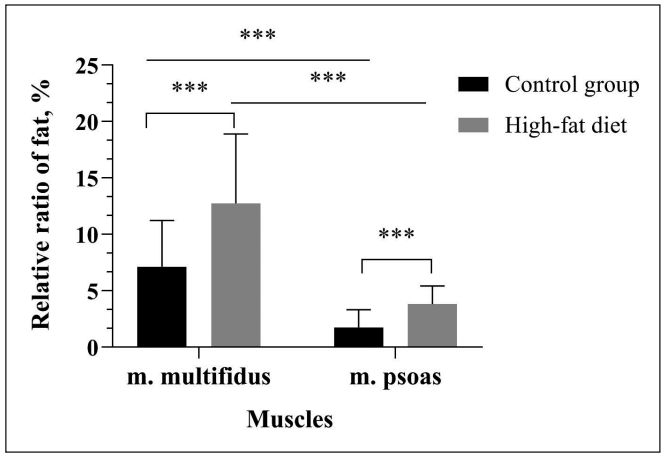

Fig. 3. Relative percentage of fat tissue in rat lumbar paraspinal muscles (m. multifidus; m. psoas) after high-fat diet. Data is shown as mean ± standard deviation. *** – p<0.001.

MATERIALS AND METHODS

The experimental study was conducted in accordance with international (Directive 2010/63/EU) and national guidelines on the care and use of laboratory animals. The plan for the experiment was approved by the local bioethics committee (protocol No. 191 from 22.04.2019).

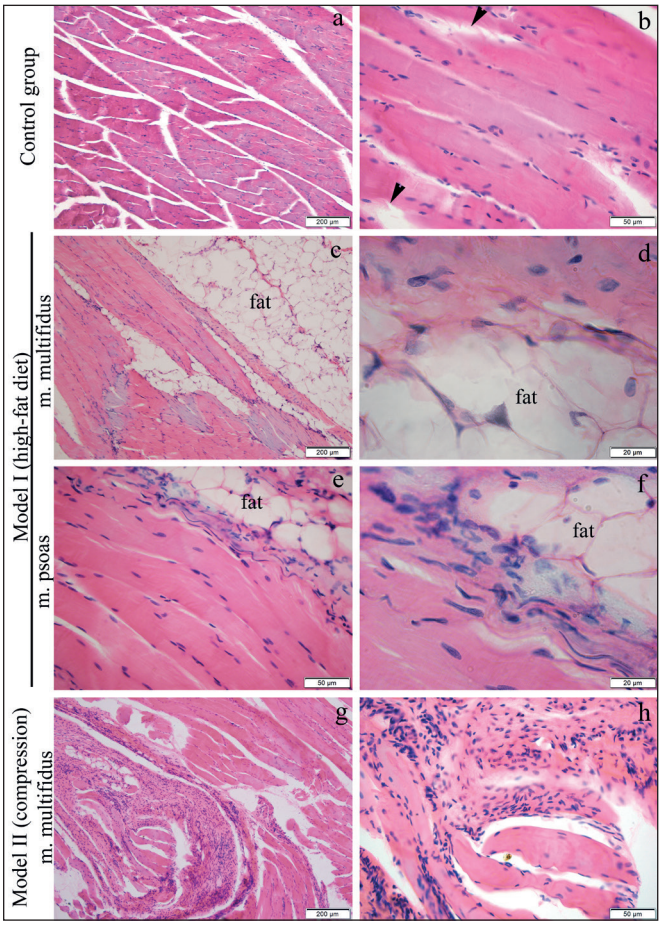

Fig. 4. Histological finding. Control group: a) polygon-shaped fibrils in the transverse section; b) striation is evident, nuclei located on the periphery of the fibres, small areas of fat (arrows). Model I (high-fat diet): c) muscle fibres replaced by fat; d) homogeneous sarcoplasm, karyopyknosis, muscle fibres replaced by adipocytes; e) muscle fibre width is uneven, 'wavy' muscle fibres, nuclei not spread out evenly; f) fragment of 2e: increased density of nuclei and adipocytes. Model II (compression): g) excess fibrous tissue growth, dystrophy of muscle fibres, increased area of fat; h) fragment of 2g: high density of fibroblasts is evident in fibrous tissue. H&E.

EXPERIMENTAL DESIGN

The study was performed on 15 white male laboratory rats (age: 2 months, weight: 140-160g) from the population of the experimental biological clinic of institution.

The creation of degenerative processes in muscle tissue of the lumbar paraspinal muscles was conducted using two models. The rats (n=15) were randomly divided into three groups of 5: control group, high-fat diet (model I), and paraspinal muscles compression (model II). For 3 months, the animals in the control group were given a normal diet without any surgical interventions. The experiment lasted for 90 days, after which all the rats were sacrificed through decapitation under ether anaesthesia.

HIGH-FAT DIET MODEL

Alimentary (diet-induced) obesity was modeled by keeping the 2-month-old rats (n=5) on a high-fat diet for 3 months. The diet was adapted from the Teklad Custom Diet TD.10670 (22.5g or 40-45% kcal from fat), which was developed by the Envigo company for laboratory rats and mice [15, 16]. The food consisted of (per 100 g): pig fat (18g), pig liver (2g), sunflower oil (3g), wheat groats (45g), cottage cheese (2g), egg powder (10g), powdered milk (6g), sugar (5g), beetroot (3g), carrot (2g), bone meal (4g). The weight of rats was measured weekly using electronic scales (BY-80, Beurer GmbH, Germany).

COMPRESSION OF THE PARASPINAL MUSCLES

The rats were anaesthetized with ketamine (50mg/kg, intramuscularly). The fur was shaved off the back, the area was treated using a Betadine solution, the rats were placed in a prone position, and a midline vertical skin incision was made to expose paraspinal muscles from Th12 to S2. The paraspinal muscles were tied from L2 to S1 with non-absorbable sutures Nurolon® 3 (Ethicon, USA) until the tied muscle became pale at each vertebral level to induce muscle ischemia. Then, the wound was sewed shut. To prevent postoperative infection, 40 mg of Gentamicin sulfate was injected into the thigh muscle immediately after the surgery (Figure 1).

HISTOLOGY

In order for a histological analysis to be performed, fragments of the lumbar m. multifidus (for both models) and m. psoas (only for the high-fat diet model) were held in 10% neutral buffered formalin for 2 days, then dehydrated in a series of isopropanols of increasing concentration, and, lastly, embedded in paraffin. Histological sections were prepared on a Reichert sledge microtome and stained with hematoxylin and eosin (H&E), and Van Gieson's stain.

The structure of the cells and intercellular matrix from the histological sections was analyzed and photographed using the BX63 light microscope (Olympus, Japan) and the CellSensDimension 1.8.1 software (Olympus Soft Imaging Solution GmbH, 2013).

HISTOMORPHOMETRY

The area of the fat and fibrous tissues in the m. multifidus and m. psoas was measured in 4 different microscope fields of view (300X magnification) on each of the 3 sections from each rat. Then, the percentage of fat (Fat%) and fibrous tissue (F%) relative to the total area was calculated.

STATISTICAL ANALYSIS

The results of the measurements of Fat% were presented as mean and standard deviation, while F% measurements were presented as median and quartiles (25^{th} and 75^{th}) for the model II. The normality of the distribution of results was verified using the Kolmogorov–Smirnov test, while the group means were compared using Student's t-test. The difference between groups was considered statistically significant if $p<0.05$. Data analysis was completed using the IBM SPSS Statistics 23 software.

RESULTS

At the beginning of the experiment, the rats from the control group and the high-fat diet group had a insignificant difference in weight. After 2 weeks, rats on a high-fat diet began to weigh more than their counterparts in the control group. 12 weeks from the beginning of the experiment, the high-fat diet rats weighed, on average, 22% (312.0±23.61 *vs.* 255.71±18.36; p=0.001) more than the control group (Figure 2).

CONTROL GROUP

In the control group, m. multifidus and m. psoas maintained normal structure (Figure 3 a, b). Small groups of adipocytes were observed between some muscle fibres (Figure 3 b). It was determined that the Fat% in the m. multifidus was 4.1 times larger than the Fat% in the m. psoas (7.13 ± 4.10 *vs.* 1.77 ± 1.55; $p < 0.001$) (Figure 4).

MODEL I (HIGH-FAT DIET)

Histologically determined that muscle fibers in the m. multifidus and m. psoas were mostly close together, however, they had different widths and had unevenly stained sarcoplasms (from lightly-basophilic to a saturated eosinophilic color). In some areas of the longitudinal sections, muscle fibres with disturbed striation were observed. Some fibres had a 'wavy' shape and others were swollen. In the transverse sections, it was discovered that most muscle fibres were polygon-shaped, although there were areas where the fibres had a round shape with flattened nuclei in a state of pyknosis. The endomysium and perimysium showed signs of swelling.

In the m. multifidus, many muscle fibres were replaced by fat tissue. The structure of nearby muscle fibres was affected: striation was lost, the sarcoplasm became homogeneous, nuclei were hyperchromatic, and chaotically distributed (Figure 3 c, d). The structural changes found in rats after high-fat diet are classified as fat dystrophy.

In the m. psoas, dystrophic changes were found, similar to the ones in the m. multifidus (Figure 3 e, f). The Fat% in the m. psoas was greater in high-fat diet group than in the control by 2.2 times (3.83±1.60 *vs.* 1.77 ± 1.55; $p<0.001$), but 3.3 times less than in the m. multifidus of the same animals (3.83 ± 1.60 *vs.* 12.76±6.14; $p<0.001$) (Figure 4).

MODEL II (COMPRESSION MODEL)

Degenerative changes were found in the lumbar m. multifidus of rats that had ischemia due to interrupted blood flow that occurred as a result partially tied lum-

bar paraspinal muscles. Uneven muscle fibre width and sarcoplasm colouring, 'wavy' and swollen fibres, loss of striation, karyopyknosis, replacement of muscle fiber with fat tissue were also observed. In some swollen fibres, nuclei migrated from the periphery to the centre of the fibre. However, some muscle fibres with an increased density of large, hyperchromatic nuclei were observed, which is evidence of repair. As opposed to the control group and the model I, in the perimysium and endomysium of rats from the model II, large areas of fibrous tissues with high fibroblast density were discovered (Figure 3 g, h). Fibrous tissue replaced muscle fibres, and the F% was 12.66% (4.59; 26.36).

DISCUSSION

This study demonstrated paraspinal muscle degeneration in rats from two experimental models: high-fat diet and lumbar paraspinal muscle compression.

It is known that a high-fat diet, which is used to model obesity [17], causes skeletal muscle oxidative stress [18], and also leads to mitochondrial dysfunction [19] and causes an imbalance between mitochondrial fission and fusion processes [20]. This, in turn, negatively affects the function and structure of muscles. In this study, disturbance of structure of muscle fibres (loss of striation, karyopyknosis, and cytoplasm destruction) was observed in rats that were kept on a high-fat diet. The replacement of muscle fiber with fat in the m. multifidus (12.76% of total area) and in the m. psoas (3.83% of total area) exceeded values from the control group by 1.8 and 2.2 times respectively. Previously, we demonstrated an interruption in the lipid exchange: an increase in the overall level of cholesterol by 1.2 times, overall lipids by 1.5 times, low-density lipoproteins by 1.2 times, triglycerides by 2 times in comparison with the control group [21]. An increase in the area of fat in the muscles we studied can be explained by a resistance to leptin which occurs in obese patients. The leptin resistance leads to a differentiation in adipocytes and the accumulation of fat in skeletal muscles [22].

The increase in the amount of fat in spinal muscles is a potential cause of pain [23]. A relationship was found between back pain, degenerative spine diseases, and the amount of fat in a specific muscle [6]. It was discovered that there is a correlation between the severity of the disease and the quantity of fat in the m. multifidus in patients who experienced back pain and degeneration of the intervertebral disc [24, 25], and also unspecific chronic back pains [26]. An increase in the amount of fat contained in the m. multifidus is linked to spondylarthrosis in patients with low back pain [8].

It should be noted that the area of fat in the m. multifidus was larger than in the m. psoas by 4.1 times in the control group and 3.3 times in the obesity group. The data we obtained matches with the results of a clinical study that involved healthy volunteers and discovered (using computer tomography) that the amount of fat in the m. multifidus was about 4 times larger than in the m. psoas [5]. Thus, the normal amount of fat in the m. psoas is less than in the m. multifidus. Similar results were obtained using computer tomography scans of patients with degenerative spine diseases [6]. It is likely that this difference becomes less significant in obese patients. According to the data from our experiment, rats that were kept on a high-fat diet had a greater increase in fat area in the m. psoas than in the m. multifidus (2.2 times vs 1.8 times) compared to the control group.

Tying the muscle leads to its deformation and ischemia. This disturbs the metabolism of the muscle, and, in cases of long exposure, causes cell death [27]. One of the reasons for this is oxidative stress, which occurs as a result of an accumulation of reactive oxygen species due to insufficient blood flow [28]. However, in more recent experimental studies, skeletal muscle injuries were mostly caused by temporary prolonged compression [29, 30] or by tying extremities to limit blood flow [28] and induce ischemia. The aim of our model is to create a prolonged but partial suppression of blood flow to the lumbar paraspinal muscles. This state is often found in people that lead a sedentary lifestyle or patients with limited mobility due to back pain. In this model, we did not observe muscle necrosis, but some muscles were replaced with fibrous tissue. Degenerative changes were found in the lumbar m. multifidus: muscle fibre swelling, loss of striation and polygonal structure, formation of fat and fibrous tissues (12.66% of the total area). In another comparable study, the authors also tied the paraspinal muscles but used more stitches, removed them after only 2 weeks, and observed the muscles 2, 6, and 10 weeks after that [10]. Analogous to our study, the authors found fibrosis in the paraspinal muscles but did not conduct histomorphometric analysis.

CONCLUSIONS

This study evaluates two models of injuring the paraspinal muscles: I — keeping rats on a high-fat diet (simulating obesity), II — creating a prolonged compression by partially tying the paraspinal muscles using non-absorbable sutures. The relevance of the models is proven: after 3 months, it is possible to obtain degenerative changes in the muscle tissue that are extremely similar to those observed in the muscles of patients with degenerative spine diseases. These models can be used in further studies to examine the impact of degenerative changes in paraspinal muscles on the structure and function of facet joints and intervertebral discs.

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REVIEW ARTICLE

LEGAL CHARACTERISTICS OF PHARMACEUTICAL ACTIVITY UNDER THE EMERGENCY CONDITIONS: PANDEMIC AND WAR

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ABSTRACT

The aim: Study of the system of pharmaceutical activity legal support in emergency conditions, namely, the period of epidemics outbreak and during the war.

Materials and methods: In the mentioned study, we analyze the state of the governmental pharmaceutical policy during the pandemic and the war in Ukraine. Legal norms and doctrinal positions of scientists regarding the above-mentioned issue are studied. The work analyzes the information presented by specialists in various scientific publications, and also uses scientific methods from a medical and legal point of view. This material is based on dialectical, comparative, analytical, synthetic, and complex research methods. This study analyzes the activities of pharmaceutical enterprises in the emergency state and the problem of ensuring patients' rights to medical care and epidemic safety. As part of the above-mentioned methods, we investigated the problems of pharmacies utilizing questionnaires. A survey of representatives of pharmacy chains, managers of purchasing pharmaceutical products and their pricing, in the cities of Kharkiv, Poltava, Kyiv, Mykolaiv and Lviv in the number of 460 specialists, shows negative trends in price gouging. This is a violation of patients' rights to affordable pharmaceutical products.

Conclusions: The pharmaceutical industry of Ukraine was not prepared in advance for extraordinary events, such as a pandemic and martial law, as well as for other natural disasters and foreseeable emergencies. This negatively affected and continues to affect the health of patients.

KEY WORDS: emergency, pharmacies` activity in wartime

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INTRODUCTION

In the context of our research, by emergency, we mean a wide range of events, including natural disasters such as infectious disease outbreaks, antimicrobial resistance, and unsafe food and water; man-made disasters such as conflicts, chemical and radiation disasters, the collapse of structures, transport accidents, lack of water and electricity, and air pollution; environmental disasters such as climate change and its consequences, including martial law. But taking into account the experience of more important events taking place recently, our research will be narrowed down to events related to the COVID infection and the martial law in Ukraine.

Undoubtedly that pharmaceutical activity is an integral part of the public health system and ensures a timely response to emergencies, including through preventive measures such as screening, vaccination, testing, medical and pharmaceutical countermeasures, as well as ensuring safety and access to medicines during natural disasters and pandemics [1], including in conditions of war [2]. In times of crisis, pharmacies are among the first-line healthcare institutions [3].

Initially, the COVID-19 pandemic became the most serious test for the health of the population, not only of Ukraine, but also of the whole world, and the continuation of this disease for patients became the so-called "post-COVID syndrome", namely when a combination of disorders at the level of several body systems leads to a persistent deterioration of health [4]. For pharmaceutical professionals, the pandemic has also become a serious test. Pharmacists are the first to expose themselves to a serious risk of infection by dispensing medicines, antiseptics, medical products, and providing pharmaceutical services. Most often, people look for help in a pharmacy before consulting a doctor. Pharmacy organizations had to adapt to new conditions. An interesting fact is that most of the vaccines against coronavirus infection during their use, at the very beginning of their introduction, did not pass full-fledged clinical trials [5] and therefore, the issue of ensuring the rights of patients when using these vaccines was on the agenda. Also, the activity of illegal Internet pharmacies, through which medicines were dispensed to patients without necessary control, became a real problem. And namely, via these illegal pharmacies, a large number of falsified products were sold [6].

Most of all the shortcomings in the medicines supply and the organization of pharmaceutical activities in Ukraine have been revealed since the beginning of the war with the Russian Federation. The fact is that pharmaceutical activity in Ukraine is excessively liberalized. Moreover, thanks to the tacit support of the state, pharmacy activity was monopolized, and most of the state and municipal pharmaceutical enterprises, including pharmacies, were

privatized. With the beginning of the Russian Federation's aggression, all the shortcomings of the state pharmaceutical policy significantly worsened the rights of patients to medical care. In addition, the outbreak of hostilities, large population movements, increased social mixing, and disruption of vaccination and surveillance services have greatly increased the risk of vaccine-preventable diseases outbreaks. Even before the war, the level of vaccination in Ukraine was the lowest in Europe [7]. There was also a problem of increasing prices for medicinal products and a shortage of certain groups of medicinal and medical products. And one can also add the complete lack of state control over pharmaceutical products imported into Ukraine and their further circulation. State authorities` attempts to take measures to improve the situation in the pharmaceutical market only create the illusion of an improvement, while at the same time imposing new problems.

Problems such as the limited availability of personal protective equipment, the high risk of infection inherent in medical professionals, and legal obstacles leading to the absence of the status of a medical services provider and related reimbursements [4], including in the conditions of war [2] are still relevant for pharmacy professionals.

THE AIM

The aim of this study was to study the system of pharmaceutical activity legal support in emergency conditions, namely, the period of epidemics outbreak and during the war. Identification of state policy shortcomings that may negatively affect the provision of the right to health of the population.

MATERIALS AND METHODS

In this study, we analyze the state of governmental pharmaceutical policy during the pandemic and the war in Ukraine. Legal norms and doctrinal positions of scientists regarding the above-mentioned issue are studied. The work analyzes the information presented by specialists in various scientific publications, and also uses scientific methods from a medical and legal point of view. This material is based on dialectical, comparative, analytical, synthetic, and complex research methods. This study analyzes the activity of pharmaceutical enterprises in the emergency, the problem of ensuring patients' rights to medical care, and epidemic safety. As part of the above-mentioned methods, we investigated the problems of pharmacies by means of questionnaires. A survey of representatives of pharmacy chains, managers of purchasing pharmaceutical products and their pricing, in the cities of Kharkiv, Poltava, Kyiv, Mykolaiv and Lviv in the number of 460 specialists, shows negative trends in price gouging. This is a violation of patients' rights to affordable pharmaceutical products.

REVIEW AND DISCUSSION

Some gaps in states' pharmaceutical policy were observed even before the COVID pandemic was declared, the consequences were not critical during the pandemic, but since the beginning of the war, all shortcomings have become more noticeable and continue to be critical. Some gaps in the legal support of states 'pharmaceutical policy became especially noticeable during the conditions of the war among them 1) the gradual liquidation of state and communal pharmacies in recent years, which led to a deterioration in the availability of medicines containing narcotic drugs and psychotropic substances, and not only in wartime; 2) tacit state support (including by local self-government bodies) for the monopolization of pharmacy activities and the creation of large pharmacy chains at both the interregional and regional level, which led to both the minimization of economic competition in the pharmaceutical market and their abuse of dominant position; 3) tacit consent of the state to the development of marketing contracts in the pharmaceutical market and, as a result, an increase in the cost of medicinal products [2].

Before researching pharmaceutical activity under martial law, it is necessary to analyze the pre-war state of legal support for pharmaceutical activity. Until February 24, 2022, in the course of the research, including a survey of pharmaceutical professionals, it was established that the prices of pharmaceutical products in Ukraine were inflated by 40-60 percent before the war due to the use of marketing agreements and exceeded the prices of similar products in individual EU countries [8].

The question arises, how can pharmaceutical marketing (which includes activities aimed at creating demand and achieving the goals of a pharmaceutical enterprise through maximum satisfaction of consumer needs in medicines and medical products) affect the negative pricing of pharmaceutical products?

The fact is that pharmaceutical marketing in Ukraine has its own "national specific". In fact, in Ukrainian realities, the task of pharmaceutical marketing is to provide services for the promotion of medicines, primarily of the manufacturer that has concluded a "marketing agreement" with the relevant pharmacy. We are even talking about prescription drugs, which are prohibited by law [8].

Pharmaceutical marketing is implemented through agreements: 1) with the participation of ordinary pharmacy chains (the parties to marketing agreements are pharmaceutical manufacturers and pharmacy activities subjects with the assistance of medical representatives and doctors); 2) with the help of digital marketing (the parties to digital marketing agreements are subjects of distance trade in pharmaceutical products (Internet pharmacies, delivery services, online pharmacies) and end consumers (patients, private medical institutions). The websites of such subjects can be located under national jurisdiction) [6].

The peculiarities of pharmaceutical marketing in Ukraine are that the Ukrainian version of pharmaceutical marketing contributed, firstly, to the improper performance of their duties by pharmaceutical and medical professionals, which consists of the promotion of certain products that are sometimes dangerous to the health of a particular patient or that do not have the necessary therapeutic effect and, as

a consequence it leads to 1) Obtaining undue benefits by pharmaceutical and medical personnel for the promotion of such products; 2) Causing harm to the patient's health.

Secondly, the monopolization of pharmacy activity, which takes place in hidden forms, results in 1) A significant increase in prices for medicines and medical products; 2) Violation of patients' right to affordable, effective medicines and medical products; 3) Falsification of pharmaceutical products.

The sale of pharmaceutical products (through legal pharmacies) was carried out according to the scheme: medical representative - doctor - pharmacist. That is, the patient does not always choose for himself what he needs, but rather what is beneficial for this relationship of persons.

As a result, a settlement scheme was developed for all doctors for pharmaceutical products recommended for purchase by patients: 1) No delivery of pharmaceutical products to the pharmacy was made without remuneration of the subject of pharmacy activity, the basis of which is a marketing agreement; 2) As a result, pharmaceutical manufacturers and importers are forced to overestimate the cost of final products.

In addition, a large market segment of retail sales from pharmacies, on the recommendation of doctors (under the guise of medicinal products) is products that are not registered as medicinal products (dietary supplements, food products for medical use, etc.).

In the course of the study, the facts of significant overpricing of certain groups of important medicinal products in comparison with the reference countries were established. It is about the principles of pricing and sale of certain groups of medicinal products to organizations financed from the budget based on the prices of medicinal products registered in the Republic of Poland, the Slovak Republic, the Czech Republic, and the Republic of Latvia, and Hungary (reference countries).

Thus, the resolution of the Cabinet of Ministers of Ukraine No. 426 of 04/03/2019 "On reference pricing for some medicinal products purchased with budget funds": health care facilities and institutions that are fully or partially financed from the state and local budgets purchase medicinal products that are included in the list, at prices that do not exceed the maximum wholesale, and retail prices set by the Ministry of Health, taking into account taxes and fees, as well as the maximum supply, sales, and trade (retail) allowances, determined by the resolution of the Cabinet of Ministers of Ukraine dated October 17, 2008, No. 955 "On measures to stabilize the prices of medicinal products".

This means that the total price for these groups of medicinal products, even when using the trade (retail) surcharge to the purchase price stipulated by the Resolution of the Cabinet of Ministers No. 955, should not exceed the price established in the Register of maximum wholesale and retail prices for some medicinal products purchased at budget funds, as of February 1, 2021, approved by order of the Ministry of Health No. 205 dated February 8, 2021.

But in the course of the research, we established the fact of purchases by pharmacies through distributors of such medicines at the following prices:

1. Diazepam injection (solution for injections) in ampoules of 5 mg/ml of 2 ml - UAH 52 per ampoule.
2. Morphine injection (solution for injections) in ampoules 10 mg / ml 1 ml - for one ampoule 70 hryvnias.
3. Fentanyl injection (solution for injections) in 0.05 mg/ml ampoules of 2 ml - UAH 64.70 per ampoule.

However, at that time, the maximum wholesale price per unit of dosage form was:

1. Diazepam injection (solution for injections) in 5 mg/ml ampoules of 2 ml - UAH 11.04 per package.
2. Morphine injection (solution for injections) in ampoules 10 mg / ml 1 ml - UAH 9.06 per package.
3. Fentanyl injections (solution for injections) in ampoules of 0.05 mg/ml of 2 ml - UAH 15.25 per package.

In the course of the survey, other facts of overpricing of pharmaceutical products, especially imported drugs, were established. For example, in EU countries Voltaren packaging with a dosage of 0.5 mg and the number of tablets - 40 pcs. cost 1.6 Euros. In Ukraine, this same drug is available in a dosage of 0.5 mg and 20 tablets, costing 284 UAH 26 kop., (which at the exchange rate of the Euro at that time was approximately 1 Euro - 30 UAH), was approximately 18 Euros for 40 tablets.

It is clear that a significant increase in the prices of pharmaceutical products occurred as a result of the massive conclusion of marketing agreements between pharmaceutical establishments. Such a situation is possible only due to the monopolization of the pharmaceutical market, its retail segment in particular.

As a result of the analysis of large pharmacy chains` activities, it was established that individual subjects of pharmacy activity operate under different brands with separate registration of the subject in each region of Ukraine, as a result of which there is an impression of maximum competition between these subjects.

This was the state of the pharmaceutical market and its legal support until February 24, 2022. What changed with the beginning of the war?

A study of the pharmaceutical market under martial law shows a number of unpredictable phenomena. These include the decrease in the number of pharmacies on the territory of Ukraine, which was connected not only with the temporary occupation of certain territories of Ukraine and/or the destruction of pharmacies but also with the usual market situation.

Thus, as of February 23, 2022, 20,800 pharmacies and pharmacy points were operating on the territory of our country. As of March 24, 2022, the number of active pharmacies was 16,700, which was 80 percent of the pre-war number of pharmacies. As of April 6, 2022, 16,200 pharmacies were already operating in Ukraine [9]. It is interesting that according to the State Service of Ukraine for Medicinal Products and Drug Control, in the first days of the war, just about 10% of pharmacies operated [10].

That is, the retail segment of the pharmaceutical market has retained its capacity. However, the very first steps of pharmacy establishments were critical for the country and patients.

What exactly is it about?

Let's start with the main problem, in our opinion, which can be corrected immediately and which we observe in the pharmaceutical market – an unjustified increase in the prices of medicinal products. At the same time, by conducting a survey, it was established that domestic pharmaceutical manufacturers did not increase selling prices.

In turn, during a survey of pharmacies representatives in Poltava, Kharkiv, Mykolaiv, Kyiv, Odesa, and Lviv regions, which belong to pharmacy networks at both the interregional level and the regional level, the fact of an increase in turnover up to 30 percent was established [11]. Interestingly, the increase in turnover is simultaneously accompanied by the optimization of the number of pharmacies. That is obvious - pharmacies are closed, sometimes for objective reasons. Although ordinary pharmacy employees themselves do not receive any additional remuneration and perform their duties professionally.

And it is worth mentioning that in conditions of increasing prices for medicinal products, the beneficiaries (owners) of pharmacy chains continue to engage in extortion from domestic drug manufacturers under the guise of marketing contracts. And all this happens in the conditions of war.

When surveying the management of domestic pharmaceutical enterprises, it was established that pharmaceutical manufacturers are forced to pay up to 60 percent of the cost of the product to interregional pharmacy chains for individual items. It is clear that these additional costs are sometimes forced by the product manufacturers to include in their own costs, reducing working capital, and, as a result, they may find themselves on the verge of bankruptcy. And this is at a time when the state needs medicine.

In this context, the appeal letter provided by pharmaceutical manufacturers, signed by the management of the "Podorozhnyk" chain of pharmacies, to the address of most pharmaceutical manufacturers, which can be considered ordinary extortion, is interesting. On the one hand, they persistently demand a discount (of course, there is no question of lowering prices), on the other hand, they warn about the need to preserve retro-bonuses[1].

Also, the letter of the "Pharmastor" pharmacy chain to the country's pharmaceutical manufacturers regarding the transfer of the pharmacy chain's debts to future payments under marketing contracts, i.e. the same retro-bonuses, is also interesting. I think it is clear how this will be reflected in the working capital of manufacturers.

That is, the final beneficiaries, the owners of individual pharmacy chains (some of them are registered in offshores) receive excess profits on the blood of our compatriots, and the end consumer (patients or the state) pays for everything.

On the one hand, the citizens of Ukraine have no other way out and are forced to buy medicine at affordable prices, on the other hand, the state in the conditions of war and maintaining combat capability bears unjustified costs in treating both wounded soldiers and providing medical assistance to its citizens.

The largest domestic distributors also took a strange position. Thus, by surveying pharmacy professionals and comparing the selling price of individual factories of pharmaceutical manufacturers and the wholesale price of the largest pharmaceutical distributors, the fact of a wholesale markup of up to 30-40 percent on certain groups of pharmaceutical products was established.

The next problem is related to the gradual liquidation of state and communal pharmacies, which is not only related to the above issue but also created other obstacles to the availability of medicines for Ukrainians.

Someone may have a question: why with such a large number of pharmacies existed before the war, was it necessary to motivate the preservation of state and communal pharmacies?

The answer is very simple. Today, the majority of drugs containing narcotic substances are dispensed only by state and communal pharmacies of economic societies, which were founded with the participation of territorial communities (that is, they have a share of the communal property). This is exactly what is happening with regard to the supply of medicines at reference prices for certain categories of the population, in particular, "Chernobyl residents". That is, the first problem faced by many patients in the first days since the beginning of the war is the issue of providing patients with drugs containing narcotics and precursors (unfortunately, the specified problem cannot be solved at the regulatory and legal level in today's conditions and, its solution is possible only after the war ends if there is the political will of individual officials and statesmanship, and not a desire to continue to solve personal financial problems.

Everyone knows that activities related to the circulation of medicinal products that contain narcotic drugs, psychotropic substances, and precursors can be carried out only in the presence of certain types of licenses (separately for production, transportation, storage, trade, etc.).

In addition, the problem is the issue of pricing of this group of drugs, which is regulated by the state, as well as storage conditions, personnel qualifications, etc., including, sometimes not quite justified attention to this type of activity by law enforcement agencies. These factors do not provide an opportunity for a quick profit and, therefore, in most cases, legalized activity related to the circulation of medicinal products containing these controlled substances is not attractive for subjects of pharmacy activity.

The next noticeable problem faced by all patients and employees of pharmacy institutions since the beginning of the war is the limited supply of drugs in regional pharmacy warehouses. Generally, most pharmacies and drug warehouses receive supplies for no more than three working days. This is justified from the point of view of economic indicators, but it is unjustified from the point of view of the social function of pharmacies if they are considered to be health care institutions and not trading points. To date, this problem has been solved in most cases, but its solution is not related to the regulatory component but is an ordinary tool in the context of making a profit (we are talking about an ordinary price increase). Although, for

example, the experience of the USA shows the creation of appropriate stocks of pharmaceutical products in case of emergencies namely and exactly by the state bodies of the relevant subjects of the federation [12, 13].

The fact is that before the artificial destruction of the state and communal network [14], the necessary groups of medicines for all positions were stored in state and communal pharmacy warehouses [15, 8, 16].

Given the experience of emergencies, particularly in the United States, the researchers believe that the importance of adopting legal language to support a 30-day supply of medication cannot be overstated, especially as the primary medical need of most disaster refugees is to replace medications that are dispensed by prescription [17].

Earlier in Ukraine, communal (regional) pharmacy warehouses kept the necessary group of medicines for emergency situations. It is clear that this situation required economic support from local self-government bodies and regulatory and legal support of state authorities.

As a result, in those regions where the communal pharmacy network was preserved (or the co-founders are territorial communities), the functions regarding the dispensing of controlled groups of medicinal products, as well as for which reference prices are set, are more or less fulfilled.

Thus, citizens of Ukraine, in the conditions of war, are reaping the fruits of excessive liberalization of the pharmaceutical market.

Meanwhile, preparing for and responding to health emergencies involving medical countermeasures often raises complex legal issues and questions among stakeholders at the state and local levels. This includes concerns about extraordinary legal powers and liability [18, 19].

The Ministry of Health of Ukraine and the Government have taken some steps to simplify the pharmaceutical activity. For example, the order of the Ministry of Health of Ukraine dated 03.03.2022 No. 406 allows the importation of medicinal products into the territory of Ukraine without complying with the requirements of part 1 of Art. 17 of the Law of Ukraine "On Medicinal Products", i.e. without their passing the state registration procedure; availability of a quality certificate of a medicinal product series; licenses for the import of medicinal products. Also, order No. 406 allows the transportation of medicinal products utilizing public transport (including those used to transport passengers) and in postal or baggage shipments, provided that the storage conditions specified by the manufacturer are observed. The order of the Ministry of Health of Ukraine dated March 7, 2022, No. 429 simplified the procedure for obtaining permission to work in pharmaceutical establishments during the period of martial law in the country. By Order No. 425 of 07.03.2022, the Ministry of Health of Ukraine granted permission to store drugs containing narcotic drugs, psychotropic substances, and precursors in all warehouses of pharmaceutical (pharmacy) institutions that have an appropriate license for the circulation of controlled substances.

The Cabinet of Ministers of Ukraine, by Resolution No. 471 of April 15, 2022, adopted a number of changes related to the procedure for state registration and re-registration of medicinal products and their circulation in wartime conditions. In particular, the Procedure for emergency state registration of medicines, medical immunobiological preparations, and blood preparations supplied to Ukraine during the introduction of martial law under obligation was approved.

However, all these measures are of a temporary nature and cannot indicate the intention to make significant changes in the state`s pharmaceutical policy.

It is interesting that the simplification of pharmacy activities and the reduction of control led to the activation of Internet pharmacies.

Meanwhile, according to WHO estimates, the distribution of counterfeit drugs through illegal Internet pharmacies is approximately 50 percent [20]. At the same time, patients are not aware of the potential dangers associated with buying drugs via the Internet - pharmacies and cannot distinguish between legal and illegal establishments.

Internet pharmacies began to emerge in the late 1990s. As of 2020, the global Internet pharmacy market, according to cyber security experts, is estimated at approximately $68.2 billion USD, and, according to forecasts, by 2027 it may grow to 202.2 billion USD. [21] That indicates that health-related technologies are undergoing an evolution driven by the digitalization of the health care system and the widespread use of the Internet in everyday life.

Digital forms of pharmaceutical marketing aimed directly at the consumer have become globalized in the era of free and open exchange of information [22]. Some researchers agree that the new direction of pharmaceutical services related to digitalization, offered by Internet pharmacies, is very attractive to most patients, especially those with limited physical capabilities [23]. This is the possibility of online orders within 24 hours, the economic availability of most pharmaceutical products, and the issue of confidentiality. For example, there is an increased demand for contraceptives in Internet pharmacies [22]. However, the Internet differs from other media in at least one important respect: it allows buyers from all over the world to shop with relative anonymity on a 24-hour marketplace.[22].

CONCLUSIONS

The above gives reasons to conclude that the pharmaceutical industry of Ukraine was not prepared in advance for extraordinary situations, such as a pandemic and martial law, as well as for other natural disasters and foreseeable emergencies. This negatively affected and continues to affect the health of patients. In order to effectively eliminate all threats in the field of pharmaceutical activity the delivery of pharmaceutical preparations and medical items, along with measures to ensure the proper use of products whose stocks are limited should be the top priority. However, taking into account the results of the studies mentioned above, it seems that the owners of the pharmacy enterprises of Ukraine, with the tacit consent of the authorities, con-

sider the highest priority to receive excess profits. Even in emergencies.

Despite the positive experience of most EU countries that had developed a lot of documents emphasizing the role of pharmacies and their contribution to responding to emergencies. Most of them are aimed at providing a comprehensive list of types of activities, separately emphasizing those necessarily related to the functions of medical supply. But other activities that are generally considered necessary to ensure a reliable emergency response (such as arranging alternative care for dependents and ensuring timely vaccinations among liquidators), may not be indicated or demonstrated less prominently, and thus are likely to be reduced or get a lower priority in pharmacists' collaborative efforts to ensure emergency preparedness [24].

Therefore, the pharmaceutical policy of the state, despite its failed nature, which led to the deterioration of the medical supply during the martial law period, has not changed and continues to contribute to the deterioration of citizens' rights to affordable and effective pharmaceutical products. Moreover, the promotion of the monopolization of pharmacy activity and the receipt of illegal income by the beneficiaries of pharmacy chains continues. At the same time, the experience of EU countries regarding the demonopolization of the pharmacy market is not taken into account.

For comparison, in the EU countries, it is a common practice to acquire the right of ownership of no more than four pharmacies for one pharmacist. Meanwhile, in Ukraine, the average number of pharmacies is from 500 to 1,000 within the framework of one subject of pharmacy activity. This requires the development of a separate draft law on pharmaceutical activity, as is the case in some European countries. In addition, it is advisable, within the framework of legal support, to create conditions for increasing the stability of medical supply. In this regard it is necessary to create regular pharmacies, ready to work in continuous mode, protected from external damage, have a register of pharmacists admitted working in wartime, and whose medicines stocks are meant to be ready in case of an emergency. Different options are possible, with good potential for partnership, in which the state is responsible for the norms and specifications of drug stocks and bears financial responsibility for ensuring the availability of a minimum stock of essential drugs.

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REVIEW ARTICLE

SPICES: MODERN VIEWS ON THE APPLICATION THROUGH THE PRISM OF POLY- AND COMORBIDITY OF PATIENTS AND INFECTIOUS PANDEMICS (LITERATURE REVIEW AND DISCUSSION)

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ABSTRACT

The aim: To substantiate the need of wider use of species in dietician rehabilitation of patients with poly- and comorbidity, postcovid syndrome based on the analysis of the latest scientific achievements with the study of their pharmacological properties.

Materials and methods: The information search in printed and electronic editions, search scientific bases with application of methods of the analysis, comparison and generalization of information data is carried out.

Conclusions: New scientific data on the pharmacological properties of spices give grounds to use them more widely in the rehabilitation of patients with poly- and comorbidities and infectious processes.

KEY WORDS: spices, pharmacological properties, polymorbidity, comorbidity, application

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INTRODUCTION

The second half of the twentieth century and the beginning of the XXI century are marked by a certain change in the spectrum of diseases of the peoples of the planet: mainly infectious diseases of the last century are replaced by metabolic diseases [1]. Among the latter, obesity has been recognized as the main non-infectious pandemic of recent decades, affecting 20-25% of the population of European countries and up to 35% of the adult population of the United States [2] and has a tendency to increase further. It is considered the «cradle» of the ten most socially significant diseases: hypertension, various forms of coronary heart disease, including myocardial infarction; strokes, type 2 diabetes, fatty liver disease, calculous cholecystitis, pancreatitis, osteoarthritis and various forms of cancer [3]. Several of these diseases are often found in patients in the form of pathogenetic combinations (comorbidity) or against the background of other diseases, including digestive, respiratory, renal, etc. (polymorbidity).

Poly- and comorbidity are considered one of the key problems of modern medicine, which significantly complicates the strategy of treatment of such patients and increases the cost of their medical care [1, 4, 5].

Recent studies have shown that nonspecific pathogenetic basis for the development of comorbidity is oxidative stress, endothelial dysfunction and associated vascular and metabolic disorders, cytokine imbalance, systemic low-intensity inflammation and immune dysfunction [1]. A number of scientists believe that the search and additional inclusion in the treatment of such patients of various means of metabolically diverse and polysystemic effects on these nonspecific links of comorbid phenomena can improve overall treatment outcomes and reduce the progression of comorbid dependencies [6]. In this direction, a significant number of studies are currently conducted using various drugs, dietary supplements, as well as increased attention is paid to the development and application of various options for health nutrition. [2, 3, 6].

In addition to traditional approaches to the balance of basic dietary ingredients, vitamins, amino acid-micro-macroelement composition, in recent years much attention has been paid to the use of various spices [6-11]. This has been facilitated by numerous modern studies of the mechanisms of their positive effect on metabolic processes, functions of a number of organs and systems, testing in various diseases of internal organs and established very interesting facts that expand the horizons of their use. poly- and comorbidity. And another interesting fact. The COVID-19 infection pandemic and one of its lessons, the so-called postcovid syndrome, has been particularly frequent, severe, longer, and more difficult to treat in patients with poly- and comorbidities [8]. This problem is also subject to in-depth study and improvement of complex treatment and the use of various spices looks promising. [9, 10].

THE AIM

The aim of the work is to substantiate the need of wider use of species in dietician rehabilitation of patients with poly- and comorbidity, postcovid syndrome based on the analysis of the latest scientific achievements with the study of their pharmacological properties.

MATERIALS AND METHODS

An information search was carried out in printed and electronic publications, scientific search databases using methods of analysis, comparison and generalization of information data. More than 100 scientific sources that correspond to the research topic are analyzed; 42 sources were selected from them, which provide the most complete information about the most popular spices in Europe and the world.

REVIEW AND DISCUSSION

According to the literature analysis, the following the most valuable thing for health and primary or secondary prophylaxis of diseases are considered: ginger (*Zingiber* officinalis) [11-13], turmeric (*Curcuma longa*) [11, 14-16], garlic (*Allium sativum*) [11, 17], onion (*Allium cepa*) [11], parsley (*Petroselinum sativum*) [11, 18, 19], cinnamon (*Cinnamonum zeylanicum*) [11, 20, 21], basil (*Ocinum basilicum*) [11, 22, 23], rosemary (*Rosmarinum officinalis*) [11, 24], oregano (*Origanum vulgare*) [11, 25], peppers (black, paprika, cayenne – *Piper nigrum, annuum, frutescens*) [11, 26- 29], cloves (*Sizgium aromaticum*) [11, 30], dill (*Anethum graveolens*) [11], cumin (*Carum carvi*) [11, 32, 33], coriander (*Coriandrum sativum*) [11, 33, 34], anise (*Anisum vulgare*) [11, 35], cardamom (*Elatteria cardamonum*) [11, 36], black sowing (*Nigella sativa*)) [11, 37, 38], nutmeg (*Myristica fragrans*) [11, 39, 40].

Without going into the characteristics of the chemical composition of these spices, they preferred to highlight the features of the pharmacological effects on the disturbed metabolic processes or infectious agents, affected organs and systems. By analyzing and comparing this information, we looked for common or similar effects on the above phenomena that occur in patients with manifestations of poly- and comorbidity, as well as certain differences in these effects, which gave grounds for grouping spices by dominant mechanisms of their action on such processes. New information in the aspect of positive influence of spices on such actual diseases as oncological, diabetes mellitus, immune disorders, acute infectious processes as COVID-19 infection was especially important to us. Such an approach would allow the doctor to purposefully and differentiated selection of several spices with multifaceted and polysystemic actions for long-term or permanent use in dietary rehabilitation of patients with various diseases according to known pathogenetic disorders, taking into account the intensity and direction of pharmacological effects. According to the literature, common or similar properties of the above spices are: anti-inflammatory, antibacterial, antiviral, antifungal, antioxidant, antispasmodic, analgesic, hypocholesterolemic, hypotensive, immunomodulatory [6, 11, 26-30]. Some of them have antitumor, hypoglycemic, anticoagulant, antidepressant, diuretic, nootropic qualities [11, 16, 19, 22, 31, 36, 38].

In recent years, scientific papers have appeared in which groups of spices are outlined according to the dominant properties of their positive effect on certain metabolic disorders or diseases, etc. [6]. In our opinion, this approach is practically focused on optimizing the use of spices by doctors in the dietary rehabilitation of patients with various diseases. Based on the information from the analyzed scientific sources, we have made some additions and expanded the range of applications of spices in different areas of their positive impact (table I).

The table below lists the groups of spices that affect various pathological phenomena and human diseases and their ranking by potency.

As can be seen from the summary table on the mechanisms of dominant action on various metabolic, hemorheological disorders, inflammatory processes, infectious factors, affected organs and systems, as well as certain age-related manifestations of the CNS, etc., most of them have diverse metabolic and multiorgan effects. moderately important for patients with manifestations of poly- and comorbidity.

For example, consider spices that have a beneficial effect on the manifestations and course of diseases of the cardiovascular system of hypertensive, atherosclerotic origin or as a consequence of other metabolic diseases. According to the cited sources of literature in these cases are appropriate: parsley, garlic, coriander, cinnamon, onion, cloves, basil, oregano. They act as systemic regulators of metabolism, have a positive effect on lipid metabolism, kidney function, moderately lower blood pressure (cinnamon, cardamom, parsley, garlic - [11, 18-20, 36]), п improve microcirculation in tissues and organs. Clove, turmeric, cinnamon, ginger, chili pepper have antiplatelet properties from them. [12, 14, 15, 20, 28, 30].

Spices that promote recovery from acute and chronic respiratory diseases. Such properties are shown by ginger, parsley, garlic, oregano, onion, basil, chili pepper, cinnamon, fennel, cumin, coriander [see table]. They can be added to food, make tea (oregano, dill, cumin, coriander), decoctions of the roots (parsley), rubbed into the skin of the chest (water-alcohol solutions or patches of peppers), drink with red wine (cinnamon, peppers), inhale from crushed or finely chopped cloves of garlic, onion, essential oils of oregano, basil [11, 22, 23, 25]. The most effective is the combined use of these spices by different routes of delivery into the body (enterally, by inhalation, application) [11].

Spices that improve the course and prevent complications in diabetes.

Useful are cardamom, coriander, oregano, cinnamon, basil, cloves, onions, garlic, turmeric, parsley. In particular, cinnamon, cardamom, blackberry and basil have hypoglycemic properties. Oregano, coriander and garlic prevent formation of diabetic lesions of the liver and biliary tract;

Table I. Grouping of spices by the dominant mechanisms and ranking by the intensity of action on the body of a sich person

The name of the group of spices with a characteristic effect	Specific names of spices
Spices with anti-inflammatory action	Ginger, garlic, peppers, onions, turmeric, basil, parsley, oregano
Spices with antibacterial activity	Garlic, onion, ginger, oregano, cloves, coriander, parsley, turmeric, oregano, cinnamon, blackberries, basil, nutmeg
Spices with antiviral activity	Ginger, garlic, cinnamon, onion, basil, pepper, turmeric, cloves
Spices with analgesic effect	Ginger, cloves, peppers, oregano, basil
Spices with antioxidant activity	Garlic, onion, ginger, basil, cloves, nutmeg, cinnamon
Spices with hypocholestyrinemic properties	Garlic, coriander, cinnamon, oregano, cardamom, onion, nutmeg
Spices with antihypertensive action	Garlic, parsley, cinnamon, onion, cardamom
Spices with antitumor properties	Garlic, onion, basil, parsley, cinnamon, black sowing, nutmeg
Spices with hypoglycemic properties	Cinnamon, basil, cardamom, oregano, parsley, ginger, garlic, blackberry
Spices with immunomodulatory activity	Garlic, peppers, parsley, basil, cloves
Spices with antidepressant action	Cardamom, chili pepper, oregano, cloves, basil, nutmeg
Spices with anticoagulant activity	Cinnamon, cloves, turmeric, chili peppers, nutmeg
Spices with diuretic action	Parsley, rosemary, cardamom, oregano, basil
Spices-regulators of the digestive system	Dill, cumin, parsley, oregano, blackberry, cloves, basil, onion, cinnamon, ginger
Spices that improve microcirculation and reparative processes	All kinds of peppers, basil, garlic, ginger, oregano, onions
Spices that promote the recovery of the bronchopulmonary system	Ginger, parsley, oregano, basil, turmeric, peppers, onions, cinnamon, blackberries
Spices with antisclerotic orientation	Garlic, onion, oregano, cardamom, turmeric, cinnamon, cloves, basil
Spices that improve memory, concentration	Rosemary, coriander, blackberry, peppers, cloves, cinnamon, garlic, nutmeg
Spices with wind-blowing effect	Dill, cumin, coriander, oregano, basil, cloves
Spices, which have anthelmintic, anti-giardiasis properties	Garlic, peppers, onions, basil
Spices that help reduce body weight	Fennel, nutmeg, cardamom, black sowing
Spices that eliminate bad breath	Parsley (greens), cloves, cardamom, rosemary, cinnamon, ginger, nutmeg, anise

parsley, rosemary, turmeric, coriander - diabetic lesions of the genitourinary system; cardamom, garlic, cloves, oregano and coriander also prevent the occurrence and progression of dyslipidemia and atherosclerosis (see table I).

Spices that have a beneficial effect on diseases of the digestive system.

These include: ginger, turmeric, cinnamon, garlic, onion, dill, oregano, parsley, cumin, basil, rosemary, peppers. All of them primarily act by increasing the secretion of the stomach, pancreas, intestines, bile, improving digestion at all levels, regulating motility (see table I).At the same time, almost all of them have anti-inflammatory, antibacterial, antiviral, antifungal, analgesic, antispasmodic effects [see table]. Some of them have a good wind-blowing effect (fennel, cumin, basil, oregano). In general, they suppress the pathogenic microflora, helping to eliminate dysbiosis.

Resorption of the factors of these spices from the intestine further contributes to the normalization of lipid disorders in the blood, other metabolic processes, kidney function (parsley, rosemary, oregano, basil), act as antioxidants (garlic, ginger, basil, oregano), and some of them antitumor effect (garlic, onion, pepper, parsley, basil, nutmeg, blackberry) (see table I).

However, it should be remembered that spices in the period of exacerbation of diseases of the digestive system are contraindicated, and such as ginger, turmeric and pepper - even in remission of chronic calculous cholecystitis and gallstone disease [12, 13, 28].

One of the problems of internal medicine is the growing phenomenon of multidrug resistance of bacteria to antibiotics. Scientific studies have now proven the antibacterial effect on such pathogens of active factors of black sowing [37], garlic [11], cinnamon [21].

Difficult to understand treatment is secondary immunodeficiency (FD), which is usually the result of many causes, including various sources of chronic infection, long and debilitating lesions of the digestive system, malnutrition, environmental negatives and more. In the complex treatment of such patients it is advisable to use ginger, garlic, turmeric, onions, parsley, peppers, basil, cloves (see table I).It is advisable long-term (2-3 months) use of 2-3 spices in food (ginger, garlic, onion, turmeric, peppers, parsley), or with teas (ginger, cloves, basil), fruit drinks, smoothies, basil, cloves, parsley). These spices activate the activity of the main system of biological support - digestive, and suppress any foci of infection, have antioxidant, stimulat-

ing, adaptogenic effects, indirectly helping to restore the functional state of the immune system.

One of the greatest trials of humanity and medicine in the world in the last two years was the pandemic of COVID-19 infection, and its consequences in the form of the so-called postcovid syndrome (PCS) or long-covid syndrome [8]. In the majority of patients who have undergone inpatient variants of the acute period of COVID-19 infection, they are activated and become worse, more difficult to treat diseases that occurred in the evidence period. Such patients in the inpatient phase of treatment have undergone a forced aggressive drug load, often with various side effects, therefore, in the presence of a PCS clinic, they are reluctant to accept treatment recommendations with modern chemotherapeutic agents and are more inclined to use various physical, dietary or folk remedies. In this context, in the rehabilitation complex of non-drug treatment, it is advisable to consider the use of spices with well-studied by modern science mechanisms of action in various diseases.

An important argument for this consideration is the fact that the pandemic of COVID-19 infection in the countries of the Asia-Pacific region, where folk medicine is strong and traditionally used in the diet of spices, was much smaller than in Europe and the United States. Scientists from these countries have published a number of important articles in the world medical and biological press, which state that along with herbal medicines, spices such as ginger, cinnamon, and garlic were used. [9].

Scientists of these publications consider the use of herbal medicines and spices as an important key in the management and rehabilitation of patients with modern infectious diseases. [9, 10, 41].

Based on their own experience in the use of health nutrition in various diseases, summarized monographically [2] and the latest scientific information on the healing properties of spices in various diseases, the authors of this report participated in the dietary rehabilitation of patients with PCS with an emphasis on the use of spices [42]. Our first experience showed a fairly good result in mild manifestations of PCS in a relatively short period (up to two months) and in moderate manifestations of PCS in patients with inpatient variants of COVID-19 infection, including oxygen-dependent cases, on the background of poly- and comorbidity. Usually the rehabilitation period of such patients was longer (up to six months) and the intensity of the use of spices, especially ginger, turmeric, cinnamon, onions, garlic, cloves. We note the importance in the successful implementation of the rehabilitation process with the use of health food and spices, providing appropriate informational and educational support to patients and their families (home care team) and modern telecommunications monitoring to assess the effectiveness of rehabilitation or correction. Our results allow us to consider this area of rehabilitation of patients with different severity of PCS, as effective, cost-effective, promising and psychologically receptive to patients.

CONCLUSIONS

The state of population health of the Earth's population at the present stage is characterized by a progressive increase in age of poly- and comorbidity due to various external causes and improper lifestyle and nutrition. The progressive growth of oncological and endocrine pathology is also alarming. The pathophysiological basis of these phenomena is specific systemic disorders in the form of oxidative stress, endothelial dysfunction and the resulting vascular and metabolic abnormalities, systemic inflammation and immune dysfunction.

The pandemic of COVID-19 infection has become a catalyst for these phenomena and processes in the acute and postcovid period, which worsens the course and results of rehabilitation measures at all stages. Viral intoxication and forced intensive medication load in the acute period of the disease causes a certain intolerance to patients with postcovid syndrome rehabilitation with modern synthetic drugs and a greater tendency to use non-drug measures, especially health nutrition, which in such cases should be enhanced by various applications.

Numerous fundamental fundamental studies of the pharmacological properties of spices and testing their use in various diseases, including cancer, endocrine and even acute infectious diseases such as COVID-19 infection, have been conducted in various countries around the world..

According to the latest research, spices at the level of the gastrointestinal tract act as activators of impaired functions of various parts of the digestive system; after resorption from it, spice factors act as correctors of disturbed metabolic and regulatory processes, and in relation to various infectious agents - as inhibitors of their activity, which is especially important in the conditions of their growing polyresistance to antibiotics. All this together provides a qualitatively better level of functioning of the organism as a whole, even with polysystemic (poly- and comorbidity) lesions. This can be considered as one of the most natural ways to activate systemic sanogenetic mechanisms.

Thus, at the present stage of human existence, patients with poly- and comorbidities, especially in age, use spices in a wide range and consciously enter the diet as an effective way to prevent exacerbations and their progression, prolong quality and longer life. In this they should be helped by doctors, who themselves should acquire the necessary knowledge and experience in this area.

According to the world's leading scientist in the field of food biochemistry Colin T. Campbell in his advanced country - the United States for the entire period of training doctors in medical schools to master dietetics are given only 20-22 hours, which is clearly not enough at present. there is no mention at all. The new realities of human health / ill health put forward in this aspect much deeper requirements of the peculiarities of prevention. Is the situation with the training of doctors in European countries much better - the question is debatable, it most likely needs to be improved…

However, the information obtained by scientists allows to deepen the views of health professionals and ordinary

people and change the paradigm of using spices not only as enhancers of taste, quality and appearance of food, but as multifaceted optimizers of organs and systems, correctors of systemic metabolic and regulatory disorders in various diseases – prophylactic direction.

They provide a basis for wider use in clinical practice, in the rehabilitation of this category of patients.

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CASE STUDY

GROSS TOTAL RESECTION IN A RARE CASE OF OPTIC NERVE ASTROCYTOMA: A CASE REPORT

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ABSTRACT

The authors present a 3-year-old female with increasing proptosis and absent vision in the right eye. Chemotherapy had done for 3months. But her ailments lingered. The right eye exhibited severe proptosis and poor vision, whereas the left eye was normal with 20/20 vision. Preoperative MRI revealed a dumbbell-shaped tumor in the intra-orbital and intra-cranial section of the right optic nerve. A lateral supra-orbital approach was used to dissect the dumbbell-shaped tumor and the right optic nerve. No remnant of the tumor was discovered during a follow-up examination.

The case study demonstrates how to identify and treat ONA surgically. However, we need further research on optic nerve PA to gain a better understanding of their behavior. While gross total resection (GTR) is usually curative, tumors in deep locations may be unresectable and require alternative therapeutic procedures. Additionally, the case study emphasizes the importance of additional research on early detection and prevention.

KEY WORDS: glioma, pilocytic astrocytoma, optic nerve glioma, extent of resection

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INTRODUCTION

Optic nerve astrocytoma is a rare and slow-growing glioma classified as pilocytic astrocytoma (PA). It is more prevalent in children and teenagers. PA make up 1.5–3.5% of orbital tumors and 66% of optic nerve tumors. Seventy-five percent of optic pathway pilocytic astrocytomas occur in children less than 12 years old. The most prevalent locations are in children's optic nerves and young adults' optic chiasms. Pilocytic astrocytoma and neurofibromatosis (NF) I have been connected. NFI individuals are more likely to develop pilocytic astrocytoma, a tumor of the optic nerve or chiasm. NFI patients with pilocytic astrocytoma make about 15%–21% of all cases. Astrocytoma without NFI on the optical pathway is very unusual [1]. Primary brain tumors in children (0–19) and in adolescents (0–14) account for 15.4% and 17.6%, respectively, of the total. According to other studies, it occurs at a rate of 4.8 per million people per year. PA, on the other hand, may strike anybody at any time and is becoming more uncommon over time. In the cerebellum (42%), supratentorial compartment (36%), optic pathway and hypothalamus (9%) and brainstem and spinal cord (9%), PA may develop anywhere in the CNS (2%) [2,3]. Tumors of this kind are slow-growing, well-circumscribed, and do not infiltrate nearby tissues or progress to more malignant categories unless they are treated aggressively[2]. This study presents a case of 3-year-old female patient with the diagnosis of PA of optic nerve.

CASE REPORT

After a 6-month history of developing proptosis and deteriorating vision, a 3-year-old child presented to our hospital-regional center for neurology and neurosurgery in Uzhhorod with absent vision in her right eye. Her first visit to the other institution was nine months before, when an MRI revealed a tumor the shape of a dumbbell in the intra-orbital portion of the right optic nerve that extended into the intracranial. At that time, decision was to perform chemotherapy after a MRI diagnosis as an optic glioma. She had ten rounds of chemotherapy (carboplatin, vincristine) for three months but no decrease in size of tumor. The right eye was found to be severely proptosis whereas the left eye was found to be normal, with 20/20 vision. She didn't seem to be doing anything else. Before admitting to our hospital, we again perform MRI of the brain, which showed the mass extends about 5mm from optic chiasma (Figure 1).

So we have recommended surgical intervention to remove the mass to prevent its extension into optic chaisma and the hypothalamus.

A lateral supra-orbital approach used to accomplish gross complete resection. During surgery, a Mayfield head holder was used after the patient was anesthetized supine. To stimulate frontal lobe retraction, the heads were stretched to a maximum of 20 degrees and rotated 25 degrees toward the contralateral side. From the supraciliary arch, 3-4cm of skin incision was done to

Fig. 1. Axial T1-Weighted Contrast of MRI of the brain shows dumbbelled shape of tumor extends intra-orbital and intra-cranial

Fig. 2. Post-operation CT scan of Brain shows no hematoma

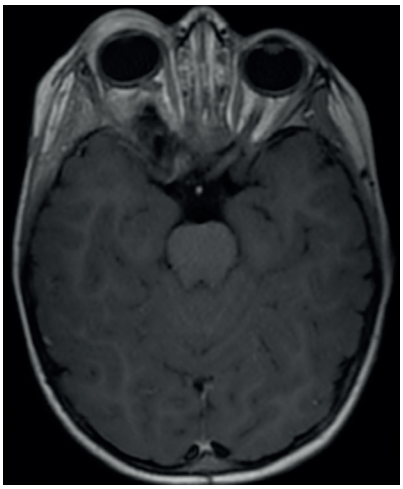

Fig. 3. First Follow up after 3month of T1-weighted MRI of the brain (axial) showed gross total resection of the tumor and no residue of tumor

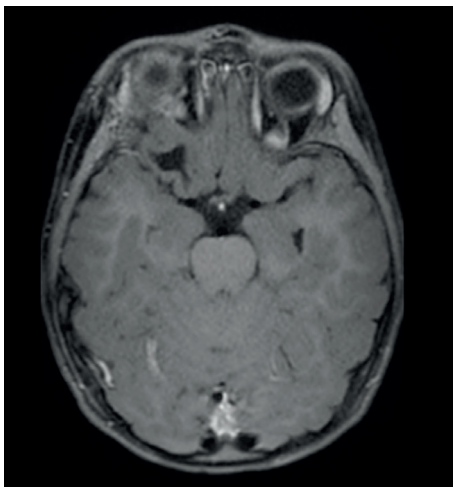

Fig. 4. One year follow up of T1-weighted contrast of MRI of the brain (axial) showed no sign of remnant of tumor

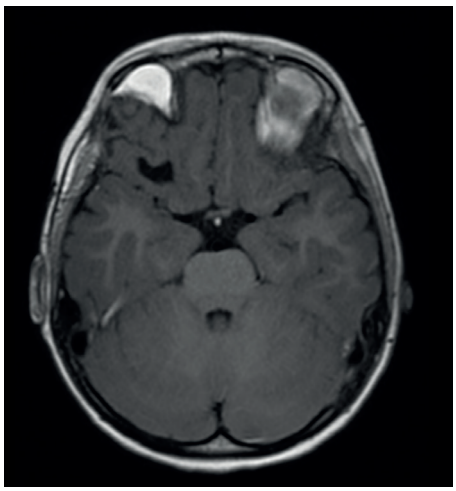

Fig. 5. The second-year followed up of T1-weighted contrast of MRI of the brain (axial)

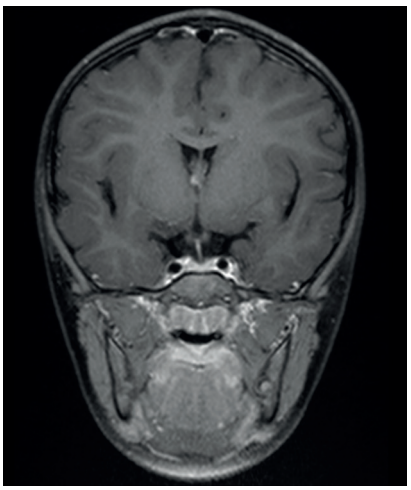

Fig. 6. The second-year followed up of T1-weighted contrast of MRI of the brain (coronal)

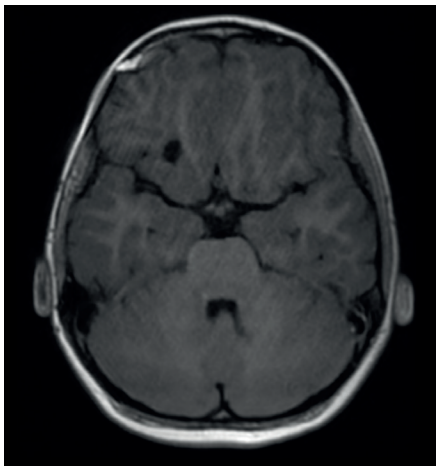

Fig. 7. Third-year followed up T1-weighted contrast of MRI of the brain (axial) shows no sign of remnant of tumor

the zygomaticofrontal suture. The orbicular muscle was pulled lower to disclose the anterior temporal line. A burr hole was drilled above the temporal line, posterior to the frontozygomatic suture. Drilling the inner border of the orbital rim near the optic nerve increased the surgical corridor and working angle. In the optic foramen of the lesser sphenoid wing. The anterior clinoid process was reached by lifting the dura immediately lateral to the optic canal. Following tumor exposure, the intracranial and intraorbital tumors were grossly resected, including the affected right optic nerve.Then step by step, the closure of skin was performed. Patient was discharged from hospital after a one week with the recommendation of a constant follow up with MRI. After 24 hours of surgery, the CT scan was performed (Figure 2) and it showed no evidence of any post-operative hematoma.

Histopathology revealed a pilocytic astrocytoma grade I. The tumor has a biphasic structure under the microscope,

with more compact sections composed of bipolar zones and highly eosinophilic Rosenthal fibers alternated with looser, spongier portions containing prominent microcysts on the microscopic level. Eosinophilic granular entities may be found in both the packed and loose zones. For the first time, the first follow-up was done after 3month, and MRI of the brain was done where no residue of tumor was seen (Figure 3).

Follow-up was made every year simultaneously to understand the status of patient, but after 3 years of follow-up, we found that there is no any sign of remnant of tumor. (Figure 4, Figure 5, Figure 6, Figure 7) and patient condition was very excellent.

Optic nerve astrocytomas are low-grade tumors that have a very diverse and surprising clinical history. They are most often seen in the optic nerve. Gliomas of the optic nerve are very uncommon and are only sometimes observed in routine clinical practice. The pilocytic astrocytoma (PA) is a benign brain tumor that often develops in infancy or early adolescence and is considered noninvasive.

These tumors usually impact the anterior optic system, causing symptoms including unilateral vision loss and proptosis. Optic nerve pilocytic astrocytomas are sporadic, like other PA. They are more common in persons with neurofibromatosis type 1. Pilocytic astrocytomas are generally indolent, regressing spontaneously after gross total resection or even without surgery.There are a variety of aggressive pilocytic astrocytomas that may extend to the brain and spinal cord, such as the pilo-myxoid type, which can return more often [3].

The tumor's topography seems to influence the disease's ocular progression. Acuity loss is more common in post-chiasmatic and optic pathway gliomas (62%) than intraorbital or pre-chiasmatic lesions (32 %). Proptosis is rare in posterior optic pathway gliomas. Absence of proptosis does not exclude saving intraorbital portions of the optic nerve. Early puberty affects 12-40% of children with chiasmatic optic pathway glioma.Lesions near the hypothalamus are thought to influence the hypothalamo-hypophyseal-gonadal axis, hence initiating puberty [4,5].

Radiologically, On CT scans, pilocytic astrocytomas often present as well-defined round/oval lesions that are iso- or slightly hypodense and significantly enhanced with contrast medium. On T1 sequences, PAs are often hypo- or iso-intense; on T2-weighted or FLAIR images, they are hyperintense [6, 7]. Vision testing, field of view measurement, ophthalmoscopic examination, and visual evoked potentials (VEP). The most reliable method is visual acuity [5].

It has the same histological and immunohistochemical characteristics as intracranial pilocytic astrocytoma. A biphasic shape, Rosenthal fibers, eosinophilic granule masses, and atypical mitotic patterns characterize both optic nerve and intracranial pilocytic astrocytomas.

Optic nerve pilocytic astrocytoma immunohistochemically positive for GFAP, OLIG2, and synaptophysin but negative for Neu-N [8,9].

Optic nerve pilocytic astrocytoma commonly appears as a cystic tumor with a mural nodule. Anatomically, a tumor is biphasic, with dense portions made up of bipolar zones and highly eosinophilic Rosenthal fibers and spongier regions with microcysts. Affected regions have eosinophilic granular bodies [2]. Consistent with the biology of pilocytic astrocytoma in all sites, the chance of recurrence is related to the surgical resection extent An great prognosis is achieved when PAs are entirely removed during surgical care [9-12].

Deep astrocytomas of the optic nerve (chiasma and brainstem) are treated with surgery, chemotherapy, and radiation. Chemotherapy should be given first in all patients with radiological or clinical progression.The treatment's side effects must constantly be handled. While multifractionated conventional radiotherapy is the most extensively used treatment, other methods of focusing radiation may be more beneficial [13, 14]. In symptomatic patients with optic pathway gliomas, imaging and follow-up visits are recommended [1].

In our case, a 3-year-old female with increasing proptosis and absent vision in the right eye was admitted. She had taken 3 months of chemo (carboplatin, vincristine) before us. But her ailments lingered. Preoperative MRI revealed a dumbbell-shaped tumor in the intra-orbital section of right optic nerve and intra-cranially extended before the optic chiasma. So, the decision was made to perform gross total resection through a lateral supra-orbital approach to dissect the dumbbell-shaped tumor and the right optic nerve to prevent the extension of the tumor to the optic chiasma so that both eye's vision would be compromised. No remnant of tumor was discovered during a follow-up on magnetic resonance imaging.

CONCLUSIONS

These uncommon pilocytic astrocytomas closely associate with neurofibromatosis type I. In our case, there was no indication of neurofibromatosis type I. Optic nerve astrocytomas grow slowly and may be managed conservatively. Optic nerve astrocytomas have a poor prognosis without therapy. Thus, surgery is only needed in cases of blindness, extreme pain, or severe proptosis. Nevertheless, all the patients should go for radiology for the evidence of extension posteriorly. When the chiasm is endangered, gross total or subtotal resection through craniotomy is essential to avoid hypothalamus or third ventricle involvement. Intracranial and intra-orbital optic nerve astrocytomas have a favorable prognosis with gross total resection and a slightly poorer after irradiation. A thorough workup, quick management, and imaging with frequent checks minimize vision loss in instances with optic nerve pilocytic astrocytoma.

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CASE STUDY

FEATURES OF THE VARIATIVE MORPHOLOGY OF THE MANDIBULAR SECOND MOLAR USING CONE-BEAM COMPUTED TOMOGRAPHY IMAGING

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ABSTRACT

The aim: To characterize the variations in the anatomical structure of the second lower molar.

Materials and methods: In order to determine the topography of the roots and root canals of the mandibular second molar, modern imaging methods were used: orthopantomography and cone-beam computed tomography.

Case report: During the analysis of the orthopantomogram of the patient, the three-root morphology of the root system of 37 teeth was revealed. That is, three roots were clearly visualized. After detecting an atypical shape of the root system of the mandibular second molar in the panoramic image, the patient was offered to perform cone-beam computed tomography (CPCT) for diagnostic purposes. Analysis by cone-beam computed tomography showed the presence of four mature roots of normal length, which were arranged in the shape of a diamond.

Conclusions: The four-root configuration is considered a rare form for mandibular molars, but it should be considered in endodontic interventions and, if possible, in the planning of such interventions, use cone-beam computed tomography.

KEY WORDS: X-ray examination, cone-beam computed tomography, Mandibular second molar, tooth clinical anatomy, root topography

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INTRODUCTION

Modern reconstructive dentistry requires a high level of knowledge of the anatomical structure of dental crowns. It is especially important to know the morphology of the masticatory surface of the teeth, as pathological lesions of hard tissues often begin in this area, as well as the topography of the roots and root canals [1-3].

Knowledge of the structure of the tooth cavity is a prerequisite for endodontic treatment, as high-quality preparation of root canals determines a positive treatment result. The greatest difficulties arise in endodontic treatment of molars due to their remote location in the dental arch and the presence of several roots, which may have different numbers of root canals of complex configuration [4, 5].

Effective restoration of defects of hard tissues of teeth with modern filling materials, as well as restoration of the integrity of the crown of the teeth with fixed prosthetic devices requires the application of this knowledge in the dentist's daily practice [6-8].

The teeth of the masticatory group are the most complex structures of the entire dental system and have a high degree of surface differentiation. Their main function, which is to grind and grind food, contributed to the emergence of a number of specific anatomical features that distinguish them from other teeth. Molars have a large area of the masticatory surface, on which there are several humps, a massive crown, which rests on two or three roots. During chewing, the teeth of the lateral group are subjected to a very significant load. It is established that the load on the first molar is approximately 77.7 kg, while on the front group of teeth - 20-40 kg. Because such a significant load is distributed over a large area due to the wide and voluminous crown and branched root system, molars are able to withstand a significant force of masticatory pressure [9-12].

The group of molars has a complex structure, significant variability in the shape and size of the crowns, as well as the position in the dental arch. The first molars are key teeth, have a stable shape and are rarely reduced. The function of the first molars is to maintain the central ratio of the jaws and stabilize the masticatory loads, protect the temporomandibular joint from compression. Keeping the first permanent molar intact is extremely important in the process of forming a permanent occlusion, it also fixes the height of the occlusion during the change of teeth, preventing the development of dental and maxillary anomalies [13-16].

The second and third molars are variable teeth, they are often characterized by signs of reduction processes, manifested by changes in the shape of crowns, their size, number of cusps, location of the main fissures, variability in the number of roots and root canals [17, 18].

Anatomical variations can be found in any tooth. Knowledge of the typical morphology and its changes helps to identify these features during endodontic interventions and increase the chance of successful treatment. The most typical anatomy of the second mandibular molar is the presence of two roots and three root canals, but other root configurations have also been described in the literature. As for the configuration with four full roots, it is the rarest, and occurs in 0.2% of cases [19].

In order to conduct a quality diagnostic process in modern dentistry, great importance is attached to the X-ray method of research. The technique of orthopantomogram research has become part of everyday practice as a mandatory stage of quality primary diagnosis. The only technique to date that allows to obtain a three-dimensional image of the study area with minimal irradiation and maximum accuracy of the obtained image is cone-beam computed tomography (CBCT) [20-23].

In today's conditions, the data of experimental study of the structure of dental hard tissues, their morphological and histological structure are significantly expanded. The study of the structural features of the structure of the hard tissues of the teeth will offer new approaches to addressing the prevention, diagnosis and treatment of carious and non-carious lesions.

THE AIM

The aim of the study is to characterize the variations in the anatomical structure of the mandibular second molar.

MATERIALS AND METHODS

This study was conducted at the orthopedic dentistry department I. Horbachevsky Ternopil National Medical University, Ukraine, and was approved by the ethics committee of the I. Horbachevsky Ternopil National Medical University, which determined that the general ethical rules of humane treatment of patients were observed when working with patients in accordance with the requirements of the Tokyo Declaration of the World Medical Association and the International Recommendations of the Helsinki Declaration of Human Rights.

In order to determine the topography of the roots and root canals, orthopantomography and cone-beam computed tomography were performed using a PICASSO Imaging system (Vatech, South Korea).

The patient was scanned in a sitting position using a special face support and centering with light rays. The frame with the sensor and the X-ray tube rotated around the patient's head by 194 degrees. For one cycle of shooting 3000 separate pictures were received, scanning time was 15-20 s. The total radiation time is 6 s.

The physical and technical parameters and the area of X-ray examination were selected on the display of the device, the final adjustment was performed using a joystick, the shooting parameters were set automatically, depending on the patient's build.

To scan the object used a plane sensor with a diameter of 24/19 cm, the generating beam was collimated in the form of a cone.

For analysis, a conical beam scan of the mandible with a slice thickness of 0.01 mm was performed. The next step was to identify areas of interest for which retrospective image reconstruction was performed in order to build the most accurate and detailed three-dimensional model. 4-6 examination zones were identified on each of the examined tomograms.

The information was processed on a computer with the operating system «Windows XP & 7» in the program «EzD2009».

After that, the three-dimensional virtual object was «cut» in layers, the appropriate thickness (0.01 mm), each slice was stored in computer memory as files in DICOM format (Digital Imaging Common Medicine).

In the process of working with the program «EzD2009» used the basic interface to work in the main option MPR (multiplanar reformation).

CASE REPORT

A 55-year-old woman of Slavic nationality was referred for a panoramic examination for the planning of dental treatment.

Orthopantomography can be considered one of the most effective additional methods of examination of patients. It gives a broad overview of the entire dental system, allows you to simultaneously see both dentitions and alveolar processes, as well as to establish the nature of interdental contacts. The technique provides high speed and ease of the procedure itself, significantly reduces the radiation dose on patients [24].

The analysis of the obtained orthopantomogram revealed an unusual morphology of the root system of 37 teeth, which looked like a three-root. That is, three roots were clearly visualized (Fig. 1).

But, as we see, even this highly informative method of examination of dental patients does not always allow to obtain all the necessary amount of diagnostic information. Therefore, after detecting an atypical shape of the root system of the second molar of the mandible on a panoramic image, the patient was offered to perform cone-beam computed tomography (CBCT) for diagnostic purposes.

Thanks to the 3D image on the CPCT, the presence of not three, as seen in the panoramic image, but four roots for tooth 37 was established. All four roots - distal, mesial, buccal and lingual - were mature, with relatively the same size and length. Moreover, both buccal and lingual roots were equivalent in size and fully developed (Fig. 2).

To determine the presence of a variation in the anatomical structure of the mandibular second molar on the opposite side, its CBCT image is considered (Fig. 3).

It is established that in this tooth there are no fully formed four roots. But the tendency to split the mesial root into three roots and canals is clearly visible.

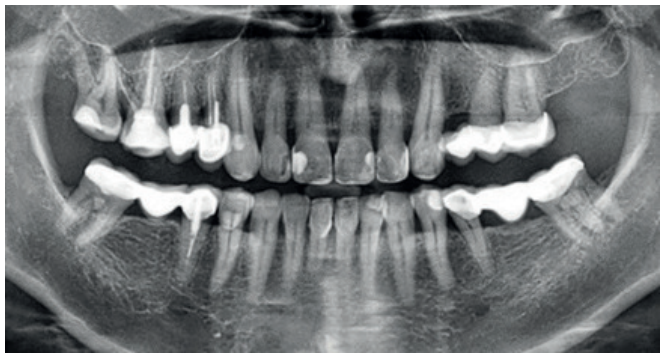

Fig. 1. Initial panoramic radiograph. Visualization of atypical anatomy of the root system of 37 tooth. 2D panoramic image shows three roots

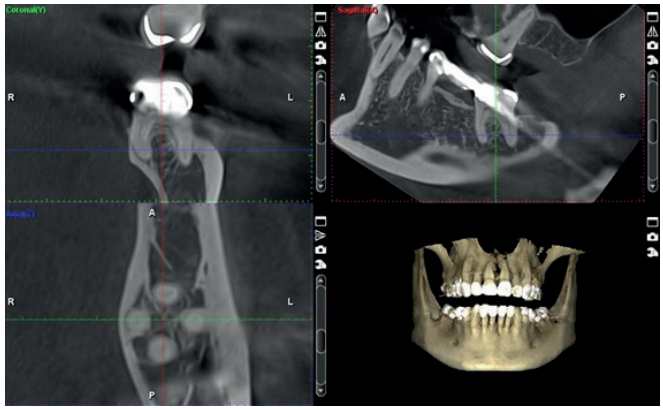

Fig. 2. Cone-beam computed tomography:
a) coronal projection performed on the buccal and lingual roots;
b) sagittal projection performed on the mesial and distal roots of 37 teeth.
c) axial projection showing the formed root system 37 of the tooth in the form of a regular rhombus.

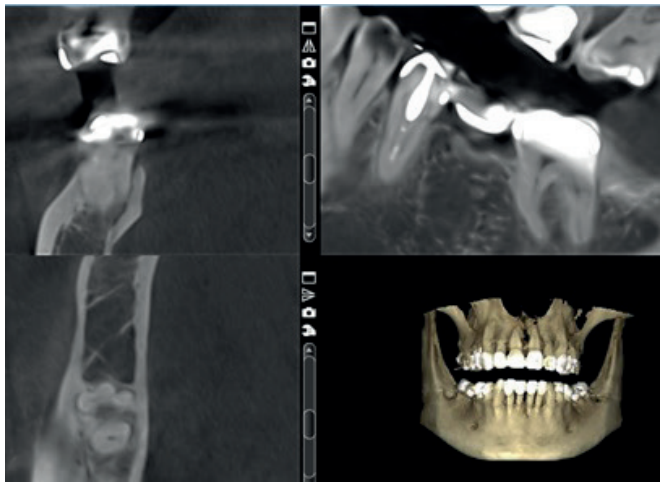

Fig. 3. Cone-beam computed tomography: axial projection. The splitting of the mesial root of 37 teeth into three parts is visualized

DISCUSSION

It should be noted that in modern dentistry the importance of the Imaging methods of research is steadily growing, which has contributed to progress in the development of computer technology.

Orthopantomography can be considered the most effective method for the primary diagnosis of the condition of the tissues of the dental-maxillary system [25, 26].

As the results of our work show in a panoramic image, the patient was found to have an atypical structure of the root system of 37 teeth. However, even this highly informative method of examination of dental patients does not always allow to obtain all the necessary amount of diagnostic information.

Unlike orthopantomography, which is characterized by image distortion of 4-7 mm, the CBCT method, with a maximum image distortion of 0.1-0.01 mm gives a more accurate detail of the clinical picture, carried out using the size of the voxel, as orthopantomography is a summation image [27, 28].

Therefore, only CBCT in this patient was visualized four-rooted system of 37 teeth.

It should be noted that the second molars of the mandible are characterized by significant variability of anatomical structure, manifested by different numbers and lengths of roots, with different direction of their curvature, as well as the presence of numerous root canal configuration options [29, 30].

According to the results of research by a large number of scientists, the mandibular second molar is characterized by significant variability in the structure of the root system. Teeth with two separate roots are common: medial and distal (73.4%). There are also forms with three roots: mainly two distal and one medial (3.8%), as well as single-rooted forms (12.6%). Regarding the number of root canals, according to scientific sources, the most common are three-channel forms (60.87%), teeth with four root canals are found in 32.3%, with five root canals - 5.7% of cases [19, 20].

As reported by the literature that root formation occurs at the embryological stages of tooth development. In multi-rooted teeth, the epithelial cells of the horizontal shell of the Hertwig's epithelial root sheath develop extensions that grow toward the center until they meet each other, dividing the original single cell into several cells, one for each root. It is known that the deposition of secondary dentin during tooth development can also lead to abnormal types of the root system of the tooth [17-20].

Therefore, it can be argued that the appearance of a four-rooted tooth means that four cells were formed as a result of intussusception of epithelial cells during root formation.

In this case, the 37th tooth has four roots: mesial, distal, buccal and lingual, which is noted as an unusual feature for the second molar of the mandible.

Thus, we can assume that the unique root system of the tooth 37 was formed by splitting the rudiment of the mesial root into three separate full-fledged roots. According to our hypothesis, during this splitting, respectively, the mesial, buccal and lingual roots of this, 37 teeth were formed, and the distal root remained unchanged. This set of factors has led to the emergence of a unique configuration in the form of a regular diamond.

CONCLUSIONS

Thus, as a result of our work, we found an extremely rare variation in the anatomical shape of the roots in the mandibular second molar of the, namely the four-root configuration. In this case, the roots are located in the shape of a regular rhombus: mesial - distal, buccal - lingual.

Therefore, when planning endodontic interventions, in order to increase their effectiveness, it is necessary to consider the above unique features of the anatomical structure of the mandibular second molar.

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