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STOOL ANTIGEN (HPSA) TEST IN DETECTION OF *HELICOBACTER PYLORI* INFECTION AMONG ADULT DYSPEPTIC PATIENTS IN TRIPOLI, LIBYA

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Background. *Helicobacter pylori is the most common infection in the world. Relationship between H. pylori and dyspepsia was confirmed by many studies, it has been strongly associated with peptic ulcer disease and gastric cancer. In that respect, several invasive and noninvasive methods for the diagnosis of H. pylori infection were utilized.*

Objective. *The aim of the study was to evaluate the association between dyspepsia and the positivity of H. pylori stool antigen test, to compare this test with serological IgG test.*

Methods. *125 adult patients were randomly selected from gastroenterology units of Mediterranean and Tajurah clinics in Tripoli. Stool samples were taken for detection of H. pylori antigen by enzyme immunoassay. Blood samples for detection of anti-H. pylori IgG antibodies were taken. Data were statistically analyzed using SPSS.*

Results. *125 dyspeptic patients: 47 male and 78 female, aged 18-83 years old were examined. 80 patients were infected by H. pylori that was proved by a positive stool test, 88 had a positive IgG test. The prevalence was higher in the patients aged 28-47 years old. There was substantial relation to age, marital status and economic risk factors; there was no association between H. pylori and gender, sources of drinking water, living standards, smoking, family history of peptic ulcer, drug consumption, and blood groups.*

Conclusions. *Relatively high rates of detection by HpSA prove that stool testing might be a reliable, simple, inexpensive, and non-invasive alternative test ащк детестшщт of H. pylori, diagnosing active infection and confirming cure. However IgG test has a low sensitivity, specificity, and accuracy compare to the HpSA test. Thus it can be used for screening purposes.*

KEY WORDS: ***Helicobacter pylori;*** **dyspepsia; enzyme immunoassay; stool antigen test.**

Introduction

The main definitive events of our knowledge of *H. pylori* came in 1979-1982 by the basic experiments of the pathologist Robin Warren, who identified the bacterium, and Barry Marshall, who successfully cultured the bacterium. In their experiments, they emphasized the association of *H. pylori* with Gastroduodenal diseases. In the end, the World Health Organization classified *H. pylori* is a class I carcinogen in 1994 due to its definite carcinogenic potential in humans [1]. *H. pylori* is a gram-negative spiral shaped, microaerophilic, and slowly growing bacterium. *H. pylori*-colonize the human stomach mainly in the mucus layer, rarely adhere to the mucosal cells, or intracellular. It is motile due to the presence of a tuft of polar flagella; moreover, it holds an important acid resistance mechanism through the generation of urease enzyme [2]. One of the principal structural parts of *H. pylori* is the flagella; it has unipolar flagella developed to move through the mucus barrier toward the gastric epithelial surface for colonization [3]. The other structural component is the outer membrane proteins (OMPs), these surface proteins have a specific important adhesive virulence factor that can bind to receptors on the surface of the gastric mucosa. One of these factors is the *BabA* (blood group antigen-binding Adhesion) [3]. The other OMP adhesion is the Sialic acid binding Adhesion (*SabA*). *Bab A* and *Sab A* adhesions are usually linked to increased risk of Gastro duodenal diseases [2]. Lipopolysaccharide (LPS) is another its structural element. It is significant in the integrity of the cell envelope of Gram-negative bacteria; besides, it can affect the severity and chronicity of infection [4]. The most significant structure of *H. pylori* is the genome. There are

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two independent *H. pylori* genomes, that have been completely sequenced the *H. pylori* 26695 and *H. pylori* J99 genomes, although the 26695 genomes was 24 KB larger, both the J99 and 26695 genomes possessed a total (G+C)% of 39%. *H. pylori* have small genome (1,667,867 base pair for 26695 type while, it is 1.643.831 for J99 type). This genome accommodates about 1,500 genes [5]. Virulence by definition is the ability of pathogens cause disease, and factors associated with this virulence are virulence factors. *H. pylori* virulence factors that are positively correlated with gastrointestinal diseases include structural, as well as secretory components. Structural components include LPS, OMPs, flagella, and the type IV secretion system (T4SS); however, its secretory components involve ammonia by urease, cytotoxin-associated gene A (*CagA*), Vaculating cytotoxin (*vacA*), and other secretory enzymes [6]. The prevalence of *H. pylori* among adults is ~30% in the United States and other developed countries, while it is more than 80% in most developing countries *H. pylori* are usually acquired in childhood [2]. Newly published two studies in African countries, reported that the prevalence of *H. pylori* infection is yet high in Morocco and Ethiopia (75.5% and 67.7% respectively), both surveys demonstrated a significant correlation with age. Another survey in Nigerian dyspeptic patients found that the prevalence was 93.6% by the serology test. These surveys showed that the prevalence was still high in this region; however, it looked lower in the western developed world in spite of higher rates among immigrants coming from countries with a high prevalence of *H. pylori* [7]. Regarding important risk factors, it was accounted for that the prevalence of *H. pylori* was positively correlated with the living in rural areas, bad sanitary status, overcrowding, low educational level, and poor socioeconomic conditions [7]. Transmission of the infection probably occurs in multiple pathways, which may differ in various societies and age groups, [8]. It has been established that there is still contention about the actual mode of transmission of *H. pylori* infection, although Person-to-person transmission through fecal-oral or oral-oral routes has been emphasized by many studies [9]. Oral-oral route of transmission depends on the presence of *H. pylori* in the gastric juice, so could reach the oral cavity through reflux or vomitus. An early study reported that *H. pylori* can be cultivated from vomitus, as a result, *H. pylori* are potentially transmissible during gastrointestinal diseases, especially, during vomiting [9], and moreover, it has been shown that H. *pylori* could be transferred through contact between persons, particularly, during childhood. The primary path of transmission is regarded through oral-oral, fecal-oral, or gastric-oral. Nevertheless, the route of transmission appears to be different, between developing, and developed countries, because the age, race, and socioeconomic status are changeable [7]. Dyspepsia could be separated into two types: ulcer dyspepsia (UD) related to organic cause and non-ulcer dyspepsia (NUD) also known as Functional dyspepsia (FD), which is specified as chronic dyspepsia without organic disease [10]. For diagnosis, functional dyspepsia has been described that the patients higher than 55 years old, or those with alarm features such as GIT bleeding, weight loss, family history of gastric cancer, should undergo quick endoscopy to exclude organic diseases; nevertheless, in patients aged 55 years or younger with no alarm features, the clinician may consider testing and treat strategy using a non-invasive test for *H.pylori* diagnosis followed by the treatment of the *H. pylori* positive patients. It was recognized that the test and treatment option was preferable in populations with a moderate to high prevalence of *H. pylori* infection (≥10 %) [11]. The rapid HpSA test based on ICA methods was more recently developed and had been used for the detection of *H. pylori* infection before and for follow up after the treatment; nevertheless, the accuracy of the rapid HpSA test is comparable, or a slight bit lower than the standard HpSA test [12]. The most distinct indications for treatment of *H. pylori* infection are those related to duodenal or gastric ulcers regardless of their activity status; other important indications are patients with low-grade gastric B cell lymphoma and after gastric cancer resection, however, many guidelines now recommend *H. pylori* treatment in uninvestigated simple dyspepsia following noninvasive diagnosis, also recommend treatment in functional dyspepsia although, it was proved that only 5-10% of patients get benefit from such treatment [2].

Combination regimens were developed to overcome the resistance to monotherapy. While, the best option is doing antibiotic sensitivities, but it is difficult and expensive and usually not indicated unless there is a failure of the second line of the treatment regimen. The recommended treatment regimen for *H. pylori* infections are the first line regimen involving

the usage of three drugs: Omeprazole, Clarithromycin, and Amoxicillin for 7-14 days; or the use of Omeprazole, Clarithromycin, and Metronidazole for 7-14 days. The second line suggests the use of four drugs: Omeprazole, Bismuth subsalicylate, Tetracycline and Metronidazole for 14 days [2].

The **objectives** of the study were to determine the prevalence of *H. pylori* among adult dyspeptic patients in Tripoli region and to evaluate the performance of stool antigen test (HpSA) as a diagnostic method for the detection of *H. pylori* infection; to compare (HpSA) test with IgG antibody test regarding sensitivity, specificity, accuracy and their useful applications; to determine the relationship of a proposed risk factor with *H. pylori* infection in the studied group.

Methods

Research design. The work was a cross-sectional study conducted to find out the prevalence of *H. pylori* among the dyspeptic patients admitted to the gastroenterology units of the Mediterranean and Tajurah specialized clinics in Tripoli from January to April 2017. Lab-based stool examination was carried out to collect data on the prevalence of *H. pylori*. In addition to this, data on risk factors were gathered from the study population using a questionnaire survey, which was focused on associated factors contributing to *H. pylori* infection. But the serological IgG antibody test was applied to all the participants for comparison with the HpSA test.

Patients. In three months, (January to April 2017), one hundred and twenty-five different patients were selected randomly from those, who were attending gastroenterology units of the Mediterranean and Tajurah specialized clinics in Tripoli city. All patients with complaints of dyspepsia were enrolled in this study.

Sample size. Blood and stool samples were collected from all participants (125 randomly selected eligible patients) and all were interviewed to answer the questionnaire.

Sample collection. An volume of 5-gram stool samples was collected from each participant in a clean, dry, waterproof container containing no detergent, preservatives or transport media; the patients' fecal samples were either frozen for long periods (60 days) or refrigerated for short periods (48 hours). Sterile Plastic stool container and ELISA antibody kits (AccuDiag™, Diagnostic Automation, Inc., California, USA) of 100% specificity and 0.5Ng/ml sensitivity [See Appendix B] were used for the study, as well as Icebox for keeping the sample through transportation. A Deep freezer for specimen storage was prepared as well as ELISA Micro plate Reader (BIO-TEC) Germany.

Assay procedure and principle of the test. *H. pylori* antigen in stool sample was determined by the ELISA using a commercially available monoclonal antibody kit. Stool samples were diluted, added (100µl) to antibody-coated microwells and incubated for 30 minutes at room temperature. *H. pylori*-specific monoclonal antibodies were conjugated with the *H. pylori* antigens. All unbound materials were washed out (three time repeated washing process). Then enzyme conjugate was added (red color solution) and incubated for 30 minutes; after adding the enzyme, it was bound to the antibody-antigen complex. The excess enzyme conjugate was washed out also, and the Chromogenic substrate was added and incubated for 15 minutes. After the addition of the stop solution to stop the reaction, a visible yellow color reaction appeared indicating the presence of *H. pylori*. The intensity of the generated color was proportional to the amount of antigen in the sample. The results were read by a microwell reader compared in a parallel with a calibrator and controls. All the tests were performed according to the manufacturer's instructions. The diagnostic ELISA, HpSA test was used as a quantitative assay for detection of *H. Pylori* antigens in a human stool specimen. The estimation of the sample concentration depended upon the construction of a standard curve, so the standard curve absorbance (O.D 450 nm) versus concentration of stool antigen in ng/ml for the quantitative detection of the stool antigen in each sample was applied, according to the ELISA kit standard.

Serological tests for detection of IgG antibodies. Three ml venous blood was withdrawn from each participant at the time of delivering stool sample, then the blood was centrifuged at speed of 2200-2500 RPM (revolutions per minute) for 10 minutes to separate the serum that was analyzed immediately or was stored refrigerated at (4–8 °C) for up to 48 hours. For a longer storage, the serum was kept at -20 °C until analyzed for anti-*H. pylori* antibodies – IgG detection using Rapid anti-*H. pylori* kit (Advanced Quality™, InTec Products, INC, China).

Assay procedure and Principle of Rapid *H. pylori* IgG Test:

We brought the device, sample diluent, and specimens to room temperature.

After we dispensed 1-2 drops (10 µl each drop) of serum to the circular sample well of the test card using the plastic dropper.

We added two drops of sample diluent to the sample well immediately.

We interpreted test results at 15 minutes.

In positive results, we saw two lines, one of them referred to the control, while the other one referred to the infected individual.

The principle of the test used for detecting IgG antibodies in human serum or plasma is a visual, qualitative test. It was performed in a single-step procedure using one to two hanging drops of serum. The test was started with a sample applying to the sample kit and adding the provided sample diluent immediately. The *H. pylori* antigens-colloidal gold conjugate embedded in the sample pad reacted with the *H. pylori* antibody present in serum or plasma sample forming conjugate/ the *H. Pylori* antibody complex. As the mixture was allowed to migrate along the test strip, the conjugate/ the *H. pylori* antibody complex was captured by an antibody-binding protein and immobilized on a membrane forming a colored test band in the test region. A negative sample did not produce a test line due to the absence of colloidal gold conjugate *H. pylori* antibody complex. A colored central band in the control region appeared at the end of test procedure regardless of test results. This control band was the result of colloidal gold conjugate binding to an anti-*H. pylori* antibody immobilized on the membrane. The control line indicated that the colloidal gold conjugate was functional. The absence of the control band evidenced that the test was invalid. HpSA should be used as the gold standard as the test offers excellent sensitivity and specificity compare to the invasive methods, such as gastric biopsy, culture, and the rapid urease test. [10].

Statistical analysis. The Data for prevalence and distribution of *Helicobacter pylori* infection among sexes and different age groups generated from the study and the association between the prevalence of *H. pylori* infection and risk factors were determined and tabulated as Microsoft Excelled sheets and uploaded to the Statistical Package for Social Sciences (SPSS version 18) Cross-tabulation of the variables were generated. Chi-square was used to detect a statistically significant correlation between the variables.

Results

Study sample description. The survey was carried on during the period from January to April 2017. One hundred and twenty-five different dyspeptic patients were included in our study to investigate the prevalence of *H. pylori* infection and its related risk factors. During this period, all patients were interviewed, and they answered the questionnaire regarding personal information and their lifestyle. At the same time samples of stool and blood were collected for investigations. The participants were living in different geographical areas of Tripoli; approximately 53% of them were from Tajurah, 22% – Tripoli central area and 17% – Ain-zarra area.

The following tables present a concise description of the study samples.

The study population age ranged from 18-83 years old. 37.6% were males with a mean age of 40.22 years old, and 62.4% were females with a mean age of39.10 years old. The male to female ratio was 1:1.66 (Table 1).

2. Detection of *H. pylori* infection by HpSA test

Table 2 presents the rate of positivity of *H. pylori* stool antigen test in adult dyspeptic

Table 1. Division of patients with dyspepsia by age and sex

Age groups	Male No.	%	Female No.	%	Total No.	%
18-27	5	20.83	19	79.17	24	19.2
28-37	11	34.38	21	65.62	32	25.6
38-47	16	55.17	13	44.83	29	23.2
48-57	6	28.57	15	71.43	21	16.8
>57	9	47.37	10	52.63	19	15.2
Total	47	37.6	78	62.4	125	100%

Table 2. Prevalence of *H. pylori* infection by HpSA test

Stool Ag. Detection	The result of *H. pylori* stool Ag. No.	The result of *H. pylori* stool Ag. %
Positive	80	64%
Negative	45	36%
Total No.	125	100%

patients among 125 adult patients, who underwent HpSA test. In (64%) 80 patients it was positive.

3. Detection of *H. pylori* infection by IgG test

Table 3 showed that the prevalence of *H. pylori* infection by IgG was 70.4%.

Table 3. Prevalence of *H. pylori* infection by IgG test

IgG Antibody detection	The result of *H. pylori* IgG Test in No.	The result of *H. pylori* IgG test in %
Positive	88	70.4%
Negative	37	29.6%
Total No.	125	100%

4. Stool antigen testing versus serology (IgG)

4.1. Comparison of frequency HpSA test (gold standard) and IgG test.

Comparison of stool antigen detection test with serology (Table 4.1) revealed that among 80 stool antigen positive cases, 64 (80%) were positive by IgG. 24 (53%) were positive for IgG out of 45 stool antigen-negative cases. This difference was statistically significant at *P*=0.002.

4.2. Accuracy of IgG test

Table 4.2 proved that sensitivity of the IgG test was 80%, while the specificity was 46.7%, total agreement (accuracy) 68%, PPV=72.7%, and NPV=56.8%.

Table 4.1. Comparison of frequency HpSA test (gold standard) and IgG test

Test	HpSA positive No.	HpSA negative No.	Total No.	*P* value
IgG positive No.	64	24	88	0.002
IgG negative No.	16	21	37	
Total No.	80	45	125	

Table 4.2. IgG Test *HPSA Test Cross tabulation.

	HPSA TEST		Total
	Positive	Negative	
IgG test Positive No. %within IgG test %within HPSA test	64 72.7% 80.0%	24 27.3% 53.3%	88 100.0% 70.4%
IgG Test Negative No. %within IgG test %within HPSA test	16 43.2% 20.0%	21 56.8% 46.7%	37 100.0% 29.6%
Total No. %within IgG test %within HPSA test	80 64.0% 100.0%	45 36.0% 100.0%	125 100.0% 100.0%

4.3. Correlations of HpSA and IgG tests with the age, marital status, monthly income, and family number risk factors

From the table above, the significant correlation of HpSA test with the age, marital status, and monthly income is evident, while IgG was positively correlated with the marital status and family number.

5. Socio-demographic characteristics of study participants

5.1. Personal risk factors

Prevalence of *H. pylori* among some associated risk factors of the study participants (Table 5.1). The Correlation is significant at the level of 0.01 (2-tailed) regarding age (*p*=0.004) and 0.05 (2-tailed) regarding marital status risk factors (*p*=0.033). While there is no significant correlation between HpSA positivity and, gender (*p*=0.976), job (*p*=0.430) and educational level (*p*=0.819).

5.2. Effects of socioeconomic status

Table 5.2 proved that the correlation was significant at the level of 0.05 for the monthly income (*p*=0.028); however, there is no correlation between the number of people living in each household (*p*=0.086) and sources of water (*p*=0.716).

5.3. Lifestyle variables, Family history of gastroduodenal disease and drug Consumption

Table 5.3 showed that there is no significant correlation between the rate of *H. pylori* infection

Table 4.3. Pearson Correlations of the HpSA and IgG tests with some of the risk factors

Correlations		HpSa Test	IgG Test	Age of the tested group	Marital status	Monthly income
HpSA Test	Pearson Correlation	1	0.280**	-0.256**	-0.191*	-0.197*
	Sig. (2-tailed)		0.002	0.004	0.033	0.028
	N	125	125	125	125	125
IgG Test	Pearson Correlation	0.280**	1	-0.116	-0.272**	-0.090
	Sig. (2-tailed)	0.002		0.199	0.002	0.320
	N	125	125	125	125	125
Age of the tested group	Pearson Correlation	-0.256**	-0.116	1	0.483**	0.077
	Sig. (2-tailed)	0.004	0.199		0.000	0.393
	N	125	125	125	125	125
Marital status	Pearson Correlation	-0.191*	-0.272**	0.483**	1	-0.035
	Sig. (2-tailed)	0.033	0.002	0.000		0.694
	N	125	125	125	125	125
Monthly income	Pearson Correlation	-0.197*	-0.090	0.077	-0.035	1
	Sig. (2-tailed)	0.028	0.320	0.393	0.694	
	N	125	125	125	125	125
FAMILY NO.	Pearson Correlation	0.154	0.224*	0.132	-0.253**	-0.001
	Sig. (2-tailed)	0.086	0.012	0.143	0.004	0.988
	N	125	125	125	125	125

*Notes. ** – correlation was significant at the 0.01 level (2-tailed);*
** – correlation was significant at the 0.05 level (2-tailed).*

Table 5.1 Personal risk factors

Variable	Negative No. HpSA	%	Positive No. HpSA	%	*P* value
Age:					0.004
18-27	15	62.5%	9	37.5%	
28-37	12	37.5%	20	62.5%	
38-47	9	31.0%	20	69.0%	
48-57	4	19.0%	17	81.0%	
>57	5	26.3%	14	73.7%	
Sex:					0.976
Male	17	36.2%	30	63.8%	
Female	28	35.9%	50	64.1%	
Marital status:					0.033
Single	14	53.9%	12	46.2%	
Married	31	31.3%	68	68.7%	
Occupational status:					0.430
Employee	15	34.1%	29	65.9%	
Housewife	15	34.9%	28	65.1%	
Private	7	28.0%	18	72.0%	
Student	8	61.5%	5	38.5%	
Education level:					0.819
Higher	12	36.4%	21	63.6%	
Intermediate	19	33.9%	37	66.1%	
Low	14	38.9%	22	61.1%	

and smoking (p=0.357), diseased family history (p=0.131) and drug consumption (p=0.363).

H. pylori infection distribution according to the blood groups

Table 5.4 showed that there is no significant statistical correlation between the rate of *H. pylori* infection and blood groups despite higher prevalence rate among blood group O.

Table 5.2. Effects of socioeconomic status

Variable	HpSA negative No.	%	HpSA positive No.	%	*P* Value
Monthly income LD:					
<1000	23	42.6%	31	57.4%	0.028
1000-2000	20	38.5%	32	61.5%	
>2000	2	10.5%	17	89.5%	
Number of people in Household:					
1-4	10	31.3%	22	68.8%	0.086
5-8	22	31.0%	49	69.0%	
>8	13	59.1%	9	40.9%	
Sources of water for drinking:					
Piped water and gallon	9	27.3%	24	72.7%	0.716
Well	16	48.5%	17	51.5%	
Filtered water (Tahlia)	20	33.9%	39	66.1%	

Table 5.3. Lifestyle variables, family history of the gastroduodenal disease and drug consumption

Variable	HpSA Negative No.	%	HpSA Positive No.	%	*P* Value
Smoking:					
No	37	38.14%	60	61.86%	0.357
Yes	8	28.57%	20	71.43%	
Family History of Peptic ulcer or Malignancy:					
No	38	39.58%	58	60.42%	0.131
Yes	7	24.14%	22	75.86%	
Consumed drugs:					
No	39	35.14%	72	64.86%	0.363
Yes	6	42.86%	8	57.14%	

Table 5.4. *H. pylori* infection distribution according to the blood groups

Blood group	HpSA positive test No.	HpSA negative test No.	Total No.	*P* value
A	24	17	41	0.761
B	20	8	28	0.819
AB	3	0	3	
O	33	20	53	0.865
Total	80	45	125	

Discussion

The accurate diagnosis and management become a vital strategy for facing the *H. pylori* challenge because of its strong association with gastroduodenal diseases as well as its high rate of infectivity especially in the developing countries. Although many diagnostic tests have been developed for detecting *H. pylori*, all have advantages as well as disadvantages. However, Breath tests or fecal antigen tests are considered the best because of their excellent accuracy, as well as they are simple and non-invasive. In the study, HpSA was used as a proxy reference test for detection of *H. pylori* infection. As reported by many international studies, it was an accurate and a reliable test in comparison with the invasive tests and Urea Breath Test for detection of active infection of *H. pylori* pathogen [13-19]; moreover, to our best knowledge, there are no previous reports of assessing *H. pylori* infection

rates by stool antigen test in Tripoli-Libya. To avoid variations in polyclonal antibodies, we used a kit that included monoclonal antibodies specific for *H. pylori* antigens to assess the presence of *H. pylori*. Monoclonal antibodies looked more accurate than polyclonal antibodies as it was documented by many studies [20]. Our study focused mainly on determining the rate of *H. pylori* infection by HpSA test in adult dyspeptic patients of Tripoli city, moreover, evaluating some of the potential risk factors associated with *H. pylori* infection, in addition, comparing HpSA test with the serological IgG test. Overall, 125 patients were involved in this study. The results proved that the age of the studied population ranged from 18-83 years old, with a mean age (40.22) (Table 5.1). It was established that the majority of the examined patients were infected with *H. pylori* according to HpSA findings. The infection rates were increasing with age and married subjects (Table 5.6). Two non-invasive tests, HpSA test – the gold standard one, were used in this study to evaluate *H. pylori* infection as well as (IgG) test for comparison. The study proved that the stool antigen assay (HpSA) has showed promising results for detection of *H. pylori* antigen in stool samples.

Conclusions

The study proved that *H. pylori* infection was a serious health problem in the Tripoli area. The rate of *H. pylori* infection among 125 adult dyspeptic subjects undergoing Stool antigen test was relatively high (64%). Most affected patients were at the age of 28-47 years old. It was more common in married subjects than single. The most significant risk factors for *H. pylori* infection was (age, marital status, and socio-economic state). Sex, smoking, occupation, education level, number of persons in each home, water consumed, family history of peptic ulcer or gastric malignancy, drug consumption and blood group could not be considered as risk factors of *H. pylori* infection as evidenced by the results of our study in Tripoli population. Finally, HpSA test is comparatively inexpensive, noninvasive, simple and accurate test for detecting *H. pylori* in a stool sample. SATs using monoclonal antibodies are useful for primary diagnosis as well as for the assessment of eradication therapy.

Recommendations

According to the findings of the study the following recommendations can be shaped. There was a high prevalence rate of *H. pylori* infection in dyspeptic patients of this study area, so high attention should be paid by governments, local administration, and district health sector. They should contribute health education to increase the knowledge of society about *H. pylori* transmission, besides, the quality of water could be tested for *H. pylori*, and there is an urgent need to provide a well-protected and treated drinking water to the community. Creating good awareness on *H. pylori* infection and its risk factor is essential because the majority of respondents have no awareness about *H. Pylori* and its risk factors, and they use any water source without care, they possess no information about the transmission pathway of *H. pylori* and its outcome.

Additionally, this study involved only stool test and questionnaire survey, so it is recommended that further studies must be carried out using different testing methods such as Urea Breath Test or Rapid Urease Test with a high number of patients.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Goma M. Huwiage – supervision, writing – review and editing, validation, data curation, *Abdurrazag A. Nami* – conceptualization, visualization, methodology, writing original draft*, Ali Hussein Akadh* – project administration, resources, software, formal analysis, investigation, funding acquisition.

ВИЗНАЧЕННЯ *HELICOBACTER PYLORI* ЗА ДОПОМОГОЮ КАЛОВОГО АНТИГЕННОГО ТЕСТУ (HPSA) У ДОРОСЛИХ ПАЦІЄНТІВ З ДИСПЕПСИЧНИМИ РОЗЛАДАМИ У ТРІПОЛІ, ЛІВІЯ

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Вступ*: Тісний зв'язок Helicobacter pylori з диспепсичними розладами, виразковою хворобою та раком шлунка підтверджено багатьма дослідженнями.*

Мета роботи*: встановити взаємозв'язок між диспепсичними розладами, позитивними результатами калового антигенного тесту та серологічної діагностики IgG на H. pylori.*

Методи*: Обстежено 125 дорослих пацієнтів з диспепсичними розладами (які лікувалися у гастроентерологічних відділень Mediterranean та Tajurah лікарень Тріполі) за допомогою: ІФА на визначення антигенів у калі та визначення у сироватці крові IgG антитіл до H. Pylori. Статистичну обробку отриманих даних проводили за допомогою програмного забезпечення SPSS.*

Результати*: Серед 125 пацієнтів з диспепсичними розладами (47 чоловіків та 78 жінок віком від 18 до 83 років) у 80 встановлено наявність H. pylori за результатами калового антигенного тесту, у 88 осіб – позитивний серологічний тест на IgG. Серед пацієнтів з позитивним результатами переважають особи віком 28-47 років. Встановлено взаємозв'язок з такими факторами як вік, сімейний статус та економічне становище. Не було достовірно значимих взаємозв'язків між H. pylori інфекцією та статтю, джерелами питної води, стандартами життя, палінням, сімейним анамнезом виразкової хвороби, вживанням лікарських засобів та групою крові.*

Висновки*: Високий відсоток частоти визначення H. pylori за допомогою HpSA калового антигенного тесту вказує, що такий метод – надійний, недорогий та неінвазивний – може слугувати для діагностики активності інфекції та моніторингу динаміки лікування. В той час, як серологічна діагностика IgG до H. pylori через нижчу чутливість, специфічність та точність може застосовуватися у якості скринінгу.*

КЛЮЧОВІ СЛОВА: ***Helicobacter pylori;*** **диспепсія; імуноферментний аналіз; каловий антигенний тест.**

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PRIMARY LARYNGEAL ASPERGILLOSIS IN AN IMMUNOCOMPETENT PATIENT (case report)

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Background. *Aspergillus is an inherently ubiquitous, weakly pathogenic fungus causing opportunistic infections. It is very rarely localized in the larynx, although laryngeal Aspergillosis may develop in the immunocompromised patients including those with leukaemia and severe aplastic anaemia.*

Objective. *The aim of the research was to explore the primary laryngeal Aspergillosis in an immunocompetent patient thru a case report.*

Methods. *A case report of primary laryngeal Aspergillosis in an immunocompetent patient is presented.*

Results. *A male patient of 40 years old, presenting with chronic worsening hoarseness, was found to have a smooth, white spheroid submucosal growth on left vocal cord with preserved bilateral cord movements on videostroboscopy. Histopathological examination of vocal cord growth revealed squamous epithelium containing septate hyphae with acute angle dichotomous branching pattern consistent with Aspergillus. Voice improved after a four-week course of oral itraconazole 200 mg/day. Post therapy follow up of 24 months was unremarkable.*

Conclusions. *Primary laryngeal Aspergillosis develops in the immunocompetent patients. Iatrogenic, vocal abuse, occupation and lifestyle factors may be contributory. Optimal diagnosis and management mandates a high index of suspicion.*

KEY WORDS: **primary laryngeal aspergillosis; videostroboscopy.**

Introduction

Aspergillus is an inherently ubiquitous, weakly pathogenic fungus causing opportunistic infections [1]. It is very rarely localized in the larynx [2], although laryngeal Aspergillosis may develop in the immunocompromised patients including those with leukaemia and severe aplastic anaemia [2-4]. Current healthcare is witnessing a surge in emerging fungal infections due to multiple exposure to antimicrobials, which can further antimicrobial resistance [5-11]. Invasive Aspergillosis of glottic, subglottic and epiglottis have been described following steroid and radiation therapy [12-15]. There are diagnostic challenges in resource-limited facilities due to overlapping presentation [16, 17]. We report a case of primary laryngeal Aspergillosis in an immunocompetent patient.

Case Report

A 40-year-old male patient presenting to the otolaryngology clinic (Army College of Medical Sciences and Base Hospital, New Delhi 110010, India) with complaints of chronic worsening hoarseness for two months. He was found to have a smooth, white spheroid submucosal growth on the anterior surface of left vocal cord with preserved bilateral cord movements on videostroboscopy. No history of vocal abuse, laryngeal trauma, sore throat, cough, dyspnea, fever or prolonged antimicrobial intake was present. There was no history of generalized immune deficiency, leukaemia, malignant disease, diabetes mellitus or use of immunosuppressive drugs and corticosteroids. No history of diabetes, tuberculosis or malignancy was present. Neither history of tobacco abuse nor history of social drinking was present. General, systemic examination and chest X-ray revealed insignificant changes. Oral cavity, oropharynx and neck were normal. HIV serology was negative. Hemoglobin was 13.5 gm/dl, total leucocytes 7800/mm^3 with normal differential count. Renal and liver function tests were in norm. Histopathological examination of vocal cord growth revealed squamous epithelium containing septate hyphae with acute angle

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dichotomous branching pattern consistent with *Aspergillus* (Fig. 1).

The findings were seconded by PAS (Periodic Acid-Schiff) stain, Grocott's (Grocott methenamine silver (GMS)) stain and KOH (Potassium hydroxide) mount, wherein hyaline hyphae with narrow angle branching were seen. *Aspergillus fumigatus* was isolated from the vocal cord tissue consecutively on Sabouraud's dextrose agar at 22 °C and 37 °C after incubation for 48 hours. Lactophenol cotton blue tease mount from blue-green suede like surface revealed septate hyaline hyphae with columnar smooth walled uniseriate conidiophores bearing phialides and conidia over flask shaped vesicles. Thermotolerance at 55 °C was observed. This was confirmed by amplification of Internal Transcribed Spacer (ITS) of 5.8S rDNA using ITS1 and ITS4 primers. Blood cultures were negative. Voice improved after a four-week course of oral itraconazole 200 mg/day. The patient was explained about good vocal practices and oral hygiene. Post therapy follow up for 24 months revealed no significant changes.

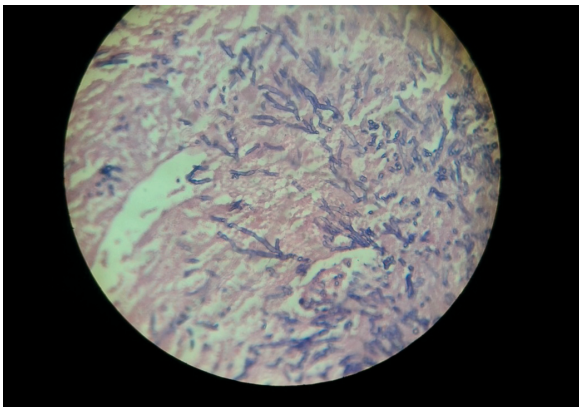

Fig. 1. Photomicrograph H&E, X400. Vocal cord growth. Squamous epithelium containing septate hyphae with acute angle dichotomous branching pattern consistent with Aspergillus.

Discussion

Approximately 50 cases of primary laryngeal Aspergillosis have been reported, half of them involved immunocompetent patients [18-24]. *Aspergillus* is non-pathogenic, or very weakly pathogenic, and causes opportunistic infections. Aspergillosis occurs due to deficient host's defense rather than fungal pathogenicity [21]. Isolated laryngeal Aspergillosis may follow colonization of larynx, which may be furthered, by local factors, rather than systemic immunosuppression. Further, systemic immunodeficiency may not contribute to the development of isolated laryngeal Aspergillosis. Iatrogenic factors such as radiation therapy, inhaled steroids and laser treatment may, also, be contributory [12-15]. Vocal abuse and oral sex may impair local protective barrier provided by healthy mucosal covering and allow colonization and subsequent invasive Aspergillosis [21]. Laryngeal Aspergillosis can even occur in a true vocal-fold cyst or laryngocoele. Systemic factors include previous prolonged antimicrobial therapy, which is implicated in altering local flora and disturbing the ecological balance between bacteria and fungi, thus allowing the growth of *Aspergillus*. Occupation, avocation and lifestyle may be contributory to exposures in healthy patients. Farmers and carpenters may be at higher risk as *Aspergillus* is a soil saprophyte. Aspergillosis had a shift in its host range as a higher incidence in males since the beginning of the 21st century has now turned to a higher incidence in females aged 20-40 years old [21].

Opportunistic infections mandate clinical intrepidity, diagnostic efficiency, appropriate timely therapy and prognostication for favourable results [25-31].

Primary Aspergillosis is often reported late due to prolonged low-grade infection and delayed onset of clinical features. Fungal growth in tissues may not corroborate to clinical presentation leaving scope for further complications such as invasion of adjacent tissues, abscess development and dissemination, for which radical surgery and aggressive antifungal therapy may be required. Fatal invasive Aspergillosis of larynx has also been reported [32].

Conclusions

Primary laryngeal Aspergillosis develops in immunocompetent patients. Iatrogenic, vocal abuse, occupation and lifestyle factors may be contributory. Optimal diagnosis and management mandates high index of suspicion.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Rajmohan K.S. – conceptualization, formal analysis, *Khan I.D.* – conceptualization, formal analysis, investigation, writing – original draft, review & editing, *Kapoor U.* – investigation, data curation, *Hashmi S.A.* – conceptualization, *Gupta R.M.* – writing – original draft, review & editing, *Sen S.* – writing – original draft, review and editing, *Nair G.L.* – data curation, *Singh K.K.* – data curation, *Tandel K.* – formal analysis, *Malik M.* – formal analysis.

ПЕРВИННИЙ АСПЕРГІЛЬОЗ ГОРТАНІ У ІМУНОКОМПЕТЕНТНОГО ПАЦІЄНТА (клінічний випадок)

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Вступ. *Aspergillus – один з найпоширеніших видів грибів з низькою патогенністю, котрий може спричиняти опортуністичні інфекції. Він надзвичайно рідко локалізується у гортані, однак може викликати аспергильоз гортані у осіб з ослабленою імунною системою, до прикладу при лейкемії чи апластичній анемії.*

Мета. *Дослідити особливості перебігу первинного аспергильозу гортані у імунокомпетентного пацієнта на прикладі клінічного випадку.*

Методи дослідження. *Описано та проаналізовано клінічний випадок первинного аспергильозу гортані у імунокомпетентного пацієнта.*

Результати. *40-річний чоловік звернувся до лікаря зі скаргами на хронічну прогресуючу захрипліcть. При огляді виявлено гладкий, білого кольору, сфероїдної форми утвір на лівій голосовій зв'язці. Рухливість зв'язок збережена білатерально, що підтверджено на відеостробоскопії. Гістологічне дослідження утвору голосової зв'язки виявило септований міцелій*

грибів роду Aspergillus (плоскоклітинний епітелій з септами з гострим кутом дихотомічного розгалуження, що відповідає аспергильозу). Покращення голосу спостерігалося після чотирьохтижневого курсу ітраконазолу, перорально в дозі 200 мг/день. При обстеженні через 24 місяці після лікування жодних відхилень не виявлено.

Висновки. *Первинний аспергильоз гортані може розвиватися у імунокомпетентних пацієнтів. Ятрогенні чинники, перевантаження голосових зв'язок, професійні шкідливі впливи та певний спосіб та стиль життя можуть сприяти його розвитку. Необхідно бути настороженим щодо можливого розвитку таких опортуністичних інфекцій для їх успішного діагностування та лікування.*

КЛЮЧОВІ СЛОВА: **первинний аспергильоз гортані; гістологічні зміни; відеостробоскопія.**

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HANSEN'S DISEASE DIAGNOSED AFTER ANTI-CANCER CHEMOTHERAPY (case report)

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Background. *Leprosy or Hansen's disease is a chronic granulomatous disease involving predominantly skin, peripheral nerves and nasal mucosa but capable of affecting any tissue or organ. Histoid leprosy is a very rare well-defined clinicopathological variant of multibacillary lepromatous leprosy, which is very difficult to diagnose due to different specific clinical and histopathological findings that mimic a fibromatous disorder. Histoid leprosy occurs generally after treatment failure and sometimes de novo.*

Objective. *The aim of the study was to explore histoid leprosy throughout a case report.*

Methods. *A case report of histoid leprosy diagnosed after cancer chemotherapy is presented.*

Results. *A 25-year-old healthy male presented with multiple skin coloured, discrete, well defined, painless papules and nodules scattered over nape of neck, right side of the trunk and both arms along with numbness as well as tingling sensation over both the arms and trunk. It was a case of non-seminomatous germ cell tumour (NSGCT), left testis, diagnosed and treated with a high inguinal orchidectomy with adjuvant chemotherapy in 2016. Ziehl Neelsen (ZN) stain for Acid Fast Bacilli (Mycobacterium leprae) – a modified Fite stain method showed numerous acid-fast bacilli. Histopathological diagnosis of Hansen's disease (Histoid) was conducted. The patient was admitted and started on triple drug multi-bacillary multi-drug therapy (MB-MDT). A remarkable improvement was noticed in the lesion status within one month of institution of the therapy.*

Conclusions. *Histoid leprosy is a discrete infrequent form of multibacillary leprosy with distinctive clinical, bacteriological and histomorphological features. Histopathologic examination with modified Fite stain is still the mainstay of diagnosis.*

KEY WORDS: **histoid leprosy; acid fast bacilli; multi drug therapy.**

Introduction

Leprosy is a chronic granulomatous disease involving predominantly skin, peripheral nerves and nasal mucosa but capable of affecting any tissue or organ. Histoid leprosy is a very rare well-defined clinicopathological variant of multibacillary lepromatous leprosy, which is very difficult to diagnose due to different specific clinical and histopathological findings that mimic a fibromatous disorder. Morphologically, histoid lepromas are sudden eruptions of dome-shaped tumours, resembling eruptive kerato-acanthomas or cutaneous metastasis.

The global burden of lepromatous leprosy is shared by Kenya, Cuba, Indonesia and Democratic Republic of Congo. India has eliminated leprosy in 2005 to less than 1 case per 10,000 population. Despite this feat achieved 13 years ago, India is the deemed leprosy capital of the world owing to a massive population of 1.32 billion. In India, Annual New Case Detection Rate (ANCDR) for leprosy is 9.71 per 100,000 population and a Prevalence Rate (PR) of 0.66 per 10,000 population with a Child Case rate of 8.94% as recorded in 2016. Pockets of high endemicity with prevalence rate of >1% are present in many parts of India [1].

Histoid leprosy has been registered to occur at an age of ten years or younger to as old as 84 years. 58% of leprosy patients are between 20 and 39 years of age with male preponderance in Indian studies [2]. Histoid leprosy is quite common in patients on irregular and inadequate dapsone monotherapy. However, the *de-novo* occurrence of the disease has only been recorded sporadically. In India, the overall incidence of histoid leprosy among leprosy patients has been estimated to be between 2.79 and 3.60% [3]. In a study from the state of Rajasthan in India, the incidence of biopsy proven histoid leprosy was 2.8% [4].

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Case report

A 25 year old healthy male, a resident of eastern Uttar Pradesh in India, presented with multiple skin coloured, discrete, well defined, painless papules and nodules scattered over nape of neck, right side of the trunk and both arms along with numbness as well as tingling sensation over both arms and trunk. The lesions were first noticed six months back with presence of a few lesions which progressed within one month, bringing him to seek medical attention. It was a case of non-seminomatous germ cell tumor (NSGCT), left testis, diagnosed and treated with a high inguinal orchidectomy with adjuvant chemotherapy in 2016.

On dermatological examination the dome shaped, nodular lesions were firm and non-tender (Fig. 1).

There was patchy hypo-aesthesia over dorsum of left hand (ulnar distribution), lower 1/3 of the anterior leg and dorsum of the foot. Non tender, uniform, peripheral nerve thickening was noted in right greater auricular, both the ulnar, both radial cutaneous, both common peroneal and left anterior tibial nerves. A healed trophic ulcer over the ulnar border of left hand was also noted. There was no loss of power or muscle wasting or deformity evidenced. His vital status and systemic examination were within normal limits.

His routine investigations such as complete hemogram, liver function test, renal function tests, chest X-ray (PA-view) were all within normal reference range. His split skin smear (SSS) showed a bacteriological index of 5+ with a morphological index of 90%. H&E stained sections from intra-lesional punch biopsy of nodular skin lined lesion showed a thin epidermis with a nodular proliferation of spindle shaped histiocytes with a clear grenz zone in the dermis (Fig. 2).

The nuclei of these fusiform cells are pyknotic with moderate cytoplasm. Occasional epitheloid component forming epitheloid granulomas also noted (Fig. 3).

Ziehl Neelsen (ZN) stain for Acid Fast Bacilli (*Mycobacterium leprae*) – Modified Fite stain method showed numerous acid-fast bacilli (Fig. 4). Histopathological diagnosis of Hansen's disease (Histoid) was performed.

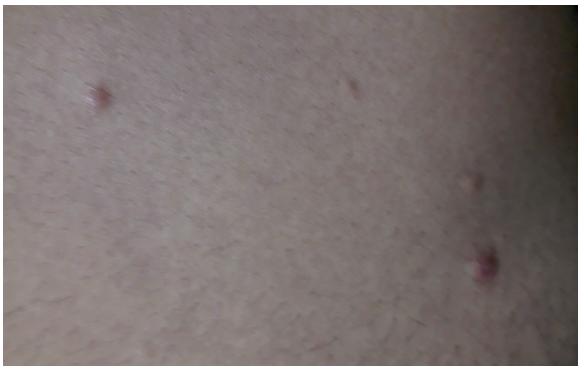

Fig. 1. Clinical presentation: Multiple soft nodules over the skin.

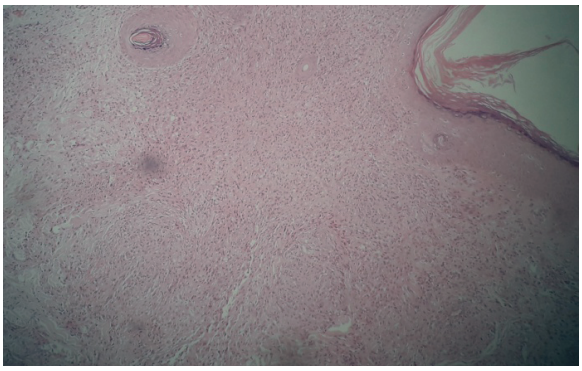

Fig. 2. Photomicrograph: H&E, X 400. Thin epidermis and nodular proliferation of spindle shaped histoid cells.

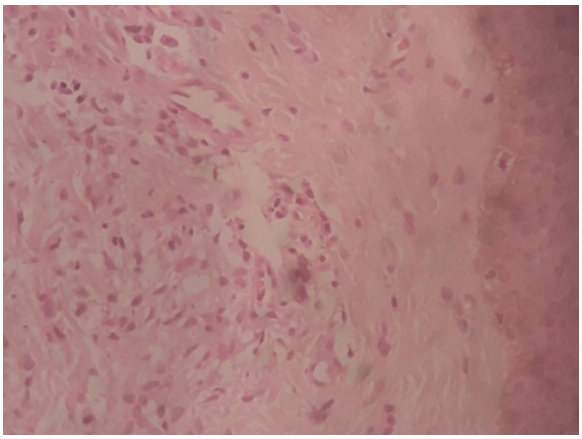

Fig. 3. Photomicrograph: H&E X 1000; demonstration of clear grenz zone.

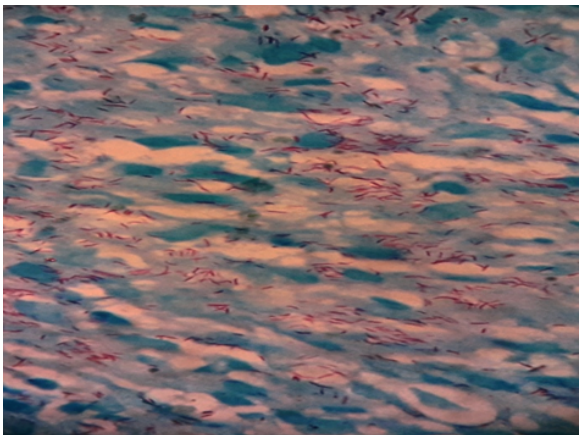

Fig 4. Photomicrograph: Fite Stain, X 1000; Fite stain showing numerous Leprae bacilli.

The patient was admitted and started on triple drug multi-bacillary multi-drug therapy (MB-MDT). A remarkable improvement was noticed in the lesion status within one month of institution of the therapy.

Discussion

Leprosy is caused by *Mycobacterium leprae* and *Mycobacterium lepromatis*, which has been discovered in 2008 causing Diffuse Lepromatous Leprosy of Lucio and Lapati in Central America and the Caribbean. Leprosy is a re-emerging disease de novo as well as in immunocompromised hosts [5]. It can present in the backdrop of immunocompromised states including tumours a seen in this patient [6].

Histoid leprosy has characteristic clinical, histopathologic and bacterial morphological features with an overall male preponderance. Macroscopically, lesions have been variously classified as subcutaneous nodules, deeply fixed cutaneous nodules, superficially placed cutaneous nodules, soft nodules, and plaques or pads over normal skin. Normally the maximum size of the lesion varies between 1.5-3 cm [7, 8]. They may have atypical presentation as giant lesions [9]. Common sites include arms, dorsum of hands, thighs, on the lower part of the back, on the buttocks and over the bony prominences, especially over the elbows and knees. Mucosal and genital lesions have been recorded in histoid leprosy [10, 11]. A single patient can have 3-50 lesions [2]. The smaller nodules are soft and the larger nodules are fibrotic. Such nodules may remain subcutaneous indefinitely or migrate towards the surface to fuse with the dermis. The patient presented almost similar kind of picture with approximately 08-10 lesions over the skin of neck, trunk and arms [12].

The classical microscopic features include epidermal atrophy as a result of dermal expansion by the underlying lepromas and an acellular band located immediately below the epidermis called sub epidermal grenz zone seen in some cases. The most striking and classical feature of typical active histoid nodules is the presence of numerous, thin, spindle-shaped histiocytes forming interlacing/intertwining bands, whorls and at times, tight curlicues giving it a tangled/storiform pattern containing acid fast bacilli. The lesion resembles a fibrohistiocytic tumour. Within the histiocytes there are numerous well-preserved acid-fast bacilli arranged in parallel bundles along the long axis of spindle histiocytes (histoid-habitus) with or without globes formation [13, 14]. The histomorphological picture in our case was in sync with histoid pattern and presence of mycobacterium confirmed on ZN for AFB (L)-modified Fite stain, which showed an abundance of the organisms.

Histoid leprosy is treated with ROM therapy followed by MB-MDT [15, 16]. High degree of suspicion is warranted for diagnosis of Histoid Leprosy, as it can be easily missed, being completely eradicated in many countries [17]. Leprosy being transmissible by direct contact is a risk in overcrowding [18]. This lesion can be misdiagnosed as a fibrohistiocytic tumour. Histological differential diagnoses include nodular sub epidermal fibrosis, dermatofibroma, and similar skin tumours on routine haematoxylin and eosin stains. Staining for acid-fast bacilli may, however, easily differentiate histoid lesions from such tumours, due to presence of Leprae bacilli in exceptionally large numbers within the fusiform cells of histoid lesions.

Conclusion

Histoid leprosy is a discrete infrequent form of multibacillary leprosy with distinctive clinical, bacteriological and histomorphological features. The appearance of histoid lesions certainly indicates a highly active lepromatous process. Histopathologic examination with modified Fite stain is still the mainstay of diagnosis along with a strong sense of suspicion.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Hashmi S.A. – conceptualization, data curation, formal analysis, investigation, writing – original draft and review & editing; *Bhadauria G.S.* – formal analysis, investigation; *Rajmohan K.S.* – conceptualization; *Khan I.D.* – conceptualization, investigation, writing – original draft and review & editing; *Gupta A.* – investigation; *Mitra D.* – investigation; *Gupta R.M.* – writing – review & editing; *Rahman M.* – investigation; *Kapoor U.* – formal analysis; *Singh S.K.* – writing – review & editing.

ХВОРОБА ГАНСЕНА (ЛЕПРА) У ПАЦІЄНТА ПІСЛЯ ПРОТИПУХЛИННОЇ ХІМІОТЕРАПІЇ (клінічний випадок)

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Вступ. *Лепра або хвороба Гансена – це хронічне гранулематозне захворювання, яке уражає переважно шкіру, периферичні нерви та слизові носа, однак може локалізуватися у будь-якому органі чи тканині. Гістоїдна лепра – рідкісний, добре описаний варіант мультибацилярного лепроматозного типу лепри, який вкрай важко діагностувати через різноманітні специфічні клінічні на патоморфологічні прояви, що подібні до фіброматозних уражень. Гістоїдна лепра як правило розвивається після невдалого та неефективного лікування, іноді de novo.*

Мета. *Дослідити особливості перебігу гістоїдної лепри, як варіанту лепроматозного типу лепри, на прикладі клінічного випадку.*

Methods. *Описано та проаналізовано клінічний випадок гістоїдної лепри, діагностованої у пацієнта після походження протипухлинної хіміотерапії.*

Результати. *25-річний чоловік звернувся зі скаргами на зміни кольору шкіри, чітко окреслені безболісні папули та вузлики, які розташовувалися навколо шиї, на правій стороні тулуба та обох руках, і супроводжувалися онімінням, поколюванням і порушеннями чутливості на обох руках і тулубі. Перед цим у нього було діагностовано негерміногенну пухлину лівого яєчка. Проведено високу пахвинну орхіектомію з наступною ад'ювантною хімотерапією у 2016 році. При забарвленні взятого клінічного матеріалу за методом Ціля-Нільсена для кислото-стійких бактерій та за модифікованим методом Файт було встановлено наявність численних кислото-стійких бактерій. При проведенні гістологічного дослідження встановлено діагноз хвороби Ганзена, гістоїдної форми. Пацієнтові призначено мульти препаратну потрійну терапію. Значне покращення стану шкіри відмічено через місяць від початку лікування.*

Висновки. *Гістоїдна лепра – рідкісне форма мульбацилярної лепри з характерними клінічними, бактеріологічними та гістоморфологічними ознаками. Гістологічне дослідження та фарбування за модифікованим методом Файт – все ще основні методи діагностики захворювання.*

КЛЮЧОВІ СЛОВА: **гістоїдна лепра; кислото-стійкі бактерії; мультипрепаратна терапія.**

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SOME METABOLIC PROCESSES IN THE PATIENTS WITH LONG-TERM CONSEQUENCES OF MILD TRAUMATIC BRAIN INJURY

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Background. *Mild traumatic brain injury (mTBI) leads to disturbance of various metabolic processes significant in pathogenesis of the maintaining of long-term consequences after it.*

The objective *of the research was to analyse changes in the activity of some membrane-associated enzyme markers, which are involved in different redox reactions, reflecting main metabolic processes.*

Methods. *Forty-seven patients with long-term consequences of mTBI, thirty controls were enrolled. The levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase were evaluated in sera by gas-liquid chromatograph and calorimetric methods.*

Results. *The study revealed significant changes in metabolic processes observed for alkaline phosphatase and LDH, which were the indicators of membrane and redox processes disturbances, acidosis severity and impaired energy cell metabolism. The averages of LDH level was 662.7 versus 381.9 U/L, in the controls. The disease progression was followed by directly proportional LDH increase reaching very high values in the patients with disease duration more than 15 years (mean ±SD 144.6±16.3 versus 82.6±8.4 U/L, controls p<0.05). The long-term consequences of mTBI were characterized by statistically significant decrease of alkaline phosphatase and positive dependence (p<0.05) of it (r=+0.48) on the disease duration with the averages of alkaline phosphatase level of 152.5±11.21 versus 212.6±9.63 U/L, controls (p<0.01). The significance of changes in membrane-associated enzymes serum levels correlated with development of oxidative stress and metabolic processes dysfunction.*

Conclusion. *In the patients with long-term consequences of mTBI, dysregulation of enzymes activity was detected that might be a marker of nervous system energy impairment and membranes destruction.*

KEY WORDS: **long-term consequences of mTBI; membrane-associated enzymes; metabolic processes.**

Introduction

Mild traumatic brain injury is a leading cause of disability in young people [2, 21]. Neurotrauma leads to disturbance of various types of metabolic processes that are significant in the pathogenesis of maintaining of long-term consequences after it [9, 12, 15]. It is established that metabolic disturbances are based on biochemical reactions proceeding with participation of enzymes located in various parts of the cell [3, 7, 22]. Most of these enzymes are inside the cell or mitochondria and appear in blood or cerebral spinal fluid only when cells are damaged, and this is of great diagnostic and prognostic importance. Thus, the changes in any parameters of membrane-associated enzymes evidence for disturbances of metabolic processes of biochemical redox reactions, cell or mitochondria destruction or development of membrane pathology [1, 4, 18, 20].

Enzymes are proteins, each type has a unique function facilitating catalyzation of routine and vital chemical reactions in the organisms [5, 17, 19]. Among the most sensitive and widely used membrane-associated enzymes are aspartate aminotransferase (AST) and alanine aminotransferase (ALT); in addition to them, alkaline phosphatase and lactate dehydrogenase (LDH) are the other interesting enzymes. LDH is not specific to the liver and can be elevated in many other diseases related to inflammation in tissues [16, 18]. Human studies have shown that serum levels of autoantibodies (anti-nuclear antibody, anti-smooth muscle antibody, and anti-liver and kidney microsomal antibody) are elevated in patients with autoimmune hepatitis and some rare muscle neurodegenerative disorders [6, 8, 10]. Symptoms of

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long-term consequences after mild traumatic brain injury can or cannot be present in the patients with mild increase of metabolic enzymes [4, 13, 21] and symptoms of posttraumatic period are not specific, thus the membrane-associated enzyme abnormalities can sometimes provide us with the useful clarification of a cause of the condition.

According to these issues, **the objective of research** was to analyze the dynamics of changes in the activity of some indicators of membrane-associated enzymes, which are involved into certain redox reactions, reflecting the main metabolic processes in the patients with long-term consequences of mild traumatic brain injury.

Methods

Forty-seven patients with long-term consequences of mild traumatic brain injury (mTBI) of the average age of 47.41±9.37 years old (16 women, 34.05% and 31 men, 65.95%) and the average disease duration (mean±SD, 12.67±8.92 years old) were enrolled into the study. The definition of mTBI was consistent with the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD-10; 1992) [2]. Thirty healthy volunteers (Control; mean±SD, 35.6±9.21 years) without neurological and psychiatry diseases were involved as well.

In the anamnesis, the investigated patients suffered from concussion (n=18; 38.29% among them there were eleven women, 61.11% and twenty men, 38.9%) and mild brain contusion (n=29; 61.71%, 17.24% women and 82.76% men). The patients were treated with symptomatic therapy. Inclusion criteria involved the patients of above 18 years of age with nonpenetrating head injury and initial Glasgow coma scale score more than 7 (mean±SD, 10.37±2.14). Exclusion criteria were craniectomy and sepsis in the anamnesis, pregnancy, preexisting neurologic diseases, acute cardiovascular diseases, respiratory failure, any acute or chronic liver diseases.

Clinical data of each case were retrieved from the patients' history. Physical and neurological examinations were performed for all patients, CT and/or MRI, EEG recording were retrieved as well. Neurological examination revealed focal neurological signs, indicating mesencephalic and brainstem structures lesions; the most frequent were tendon reflexes increase in 23 patients (41.07 %), coordination disturbances – 21 patients (37.5 %), ataxia – 15 patients (26.78 %), horizontal nystagmus – 11 patients (19.64 %), pathological foot reflexes – 8 patients (14.28 %) and rotator nystagmus – 5 patients (8.93 %).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase (LDH), creatine kinase and gamma-glutamyl transpeptidase (GGT) were detected in sera by the gas-liquid chromatography and the calorimetric methods according to the standard protocols provided by the manufacture using commercially available human ELISA kits (Clinical Biochemistry Department, Kharkiv National Medical University). Controls and individual sampling were performed in duplicate manner with a coefficient of variation of <10 % (P values ≤0.05); all protocols were approved by the local Ethics Committee; written informed consents were obtained from all patients.

Age and disease duration were compared between the groups by the χ^2 test; parametric tests were used for normally distributed data; nonparametric tests were used for abnormal distributed data; Kruskal-Wallis and Mann-Whitney U tests were applied in Prism regarding the differences between groups, the multivariate analysis considering covariates was performed.

Results

The results of all patients examinations have revealed the significant changes in membrane-associated enzymes serum levels manifested as the increase of creatine kinase (CK) serum levels by 41.4%, LDH by 73.5% and decrease of alkaline phosphatase serum levels to 28.3% and a tendency of GGT serum level decrease to 18.7% in the general clinical group (Table 1).

Analysis of the dynamics of the membrane-associated enzymes markers in the patients with long-term consequences of mTBI proved that the most severe and statistically significant changes were observed for two enzymes: alkaline phosphatase and LDH, which are the indicators of cell or mitochondria membrane damage, acidosis severity, isolation of redox processes and phosphorylation, impaired energy metabolism in cells.

Metabolic changes in the examined patients evidenced that the content of serum LDH, a key glycolysis enzyme, was increased in almost all patients, and this increase was in a direct proportion with the disease progression or longer posttraumatic period ($p<0.05$; $r=+0.36$). We found the elevated LDH level in serum

samples of the investigated patients compare to HC (p=0.01, t=5.08); in the general patient group the medians of total LDH level was 662.7 U/L and 381.9 U/L, in controls, respectively.

To evaluate the clinical prognostic value of LDH changes we further segregated among the different disease duration groups. When comparing the LDH serum level in the patients with different duration of the post-traumatic period after mTBI, it was proved that the patients with the disease duration no longer than 5 years (n=14; 29.78%) were characterized by increase of LDH serum levels by 27.8% (p>0.05). In the patient group with the disease duration from 5 to 15 years (n=11; 23.4%), the LDH was increased to 78.6% (p=0.05) with average level of 682.4±24.8 U/L, and in the patients with the disease duration more than 15 years (n=22**;** 46.8%), the LDH was increased to 105.2% (p=0.01) with average level of 783.6±31.4 U/L.

The revealed increase of LDH serum level, in our opinion, was a marker of increased membrane destruction as a result of metabolic disturbances. It should be noted that in the patients with long-term consequences for more than 15 years, LDH serum level was higher compare to HC and average group indicators, which, in our opinion, was a poor prognostic sign.

When comparing the alkaline phosphatase serum level in the patients with different duration of post-traumatic period after mTBI, it was found that the patients with the disease duration no longer than 5 years was characterized by 8.5% decreasing (p>0.05) of alkaline phosphatase serum level. In the patients with the disease duration between 5 and 15 years, the mean serum level of alkaline phosphatase was decreased by 24.1% (p>0.05); in the patients with the disease duration more than 15 years, alkaline phosphatase level was decreased by 27.6% (p<0.01) in sera.

At the same time, further disease progress or longer posttraumatic period was accompanied by directly proportional increase of CK serum levels: the values remained higher than the controls with an average of (mean ±SD) 116.8±19.4 U/L (p<0.05) in the general group and reached statistically significant values in the patients with the disease duration more than 15 years (mean ±SD 144.6±16.3 U/L versus 82.6±8.4 U/L controls; p<0.05). However, long-term consequences of mTBI revealed quite significant increase in CK level by 50.3% compare to the patients of the subgroup with the duration of the posttraumatic period less than 5 years. This correlated positively with a higher frequency of various neurological syndromes (p<0.05; r= +0.63) for long-term consequences of mTBI and might be a biochemical marker of the latter neurological deficit.

Table 1. The dynamics of the markers of membrane-associated enzymes serum levels in the patients with long-term consequences after mild traumatic brain injury

Data	All patients (general group)	Dependence from the post-traumatic period duration		
		up to 5 years (stage 1)	from 5 to15 years (stage 2)	more than 15 years (stage 3)
AST, (U/L)	29.6±3.3 p>0.05	26.6±2.9 p>0.05	27.3±4.8 p>0.05	30.3±7.1 p>0.05
Controls	25,2±1,9			
ALT, (U/L)	22.8±3.9 p>0.05	21.8±4.2 p>0.05	21.4±2.1 p>0.05	23.5±3.7 p>0.05
Controls	20,5±1,4			
Alkaline phosphatase, (U/L)	152.5±11.21 p<0.01	194.6±6.7 p>0.05	161.3±15.3 p>0.05	153.9±9.8 p<0.01
Controls	212,6±9,63			
LDH, (U/L)	662.7±21.9 p=0.01	488.3±36.3 p>0.05	682.4±23.1 p=0.05	783.6±18.4 p=0.01
Controls	381.9 ±28,1			
CK, (U/L)	116.8±19.4 p<0.05	109.5±27.1 p>0.05	126.4±22.6 p>0.05	144.6±16.3 p<0.05
Controls	82,6±8,4			
GGT, (U/L)	14.8±6.2 p>0.05	19.6±3.5 p>0.05	15.9±9.3 p>0.05	14.1±4.2 p>0.05
Controls	18,2±1,4			

Note. p – significance compared to the control group.

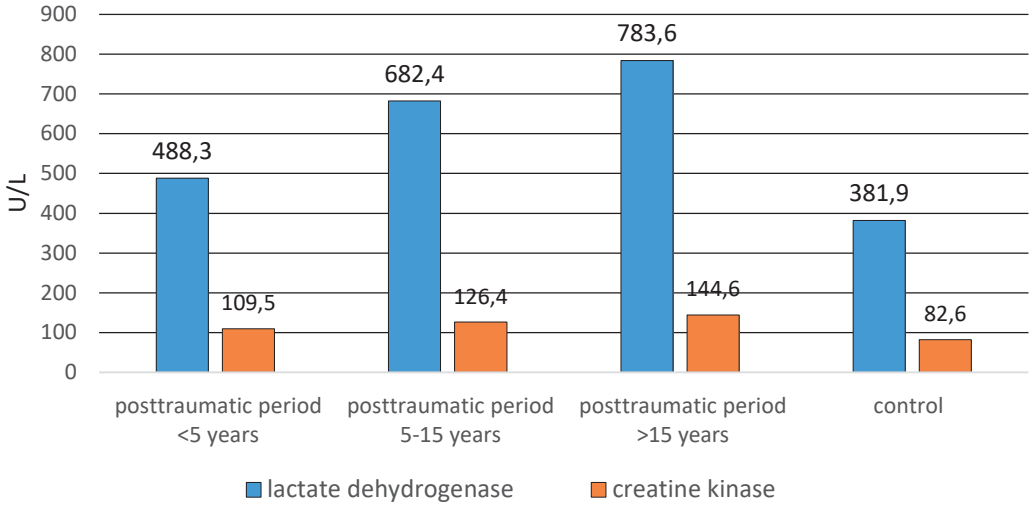

Figure 1. Analysis of the dynamics of lactate dehydrogenase and creatine kinase levels in the patients after traumatic brain injury with different duration of posttraumatic period.

The same dynamics was evidenced with the respect to GGT serum level but without statistically significant changes: the GGT serum level at stage 1 and stage 2 practically did not differ from the control. In the remaining group of the patients, there was a decrease of GGT serum levels compare to HC (mean ±SD 14.1±4.2 versus 18.2±1.4 U/L) but also without any statistically significant changes ($p>0.05$).

Discussion

The attained data complied with the literature, in our opinion reflecting the effect of the increasing deficiency of oxidative reactions in the body on aminoacid metabolism in this category of patients [11, 12, 14]. The decrease in some markers of membrane-associate enzymes activity could be a marker of impaired metabolic processes following long-term consequences after mTBI. The revealed increase of membrane-associate markers enzymes in the patients with long-term consequences after mTBI was directly proportional to the severity of neurological deficit ($p<0.05$; $r= +0.63$) as a result of the development of membrane cell or mitochondrial membrane pathology and that might be a marker of the degree of nervous system damage. In turn, the destruction of cell membranes was pathogenetically associated with the development of oxidative stress [6, 15, 16, 18].

The long-term consequences after mTBI was characterized by the statistically significant decrease of alkaline phosphatase serum level and positive dependence of alkaline phosphatase ($p<0.05$; $r=+0.48$) on the disease duration reflecting the specificity of the revealed changes in its activity in cases of cell energy deficiency in this category of the patients, which was an important diagnostic criterion. The decrease in alkaline phosphatase activity according to the previous literature [7, 10] might be a marker of impaired intracellular metabolism in long-term consequences of mTBI.

In the general patient group, there was a significant increase in CK serum level compare to controls with the maximal biochemical values in the third clinical group with long-term consequences of mTBI more than 15 years and combined in 18.2% of the patients (n=4) with partial epileptic seizures. In our opinion, the direct proportional increase in CK activity with the progression and increase of posttraumatic period was associated ($p<0.05$; $r= +0.63$) with development of destructive processes in cells as a result of oxidative stress and disturbance of intracellular bioenergetic processes. It was noteworthy that the CK increase in cases of posttraumatic encephalopathy in the patients with long-term consequences more than 15 years did not reach the same value (according to the literature) as in hereditary neuromuscular diseases and this might be a differential diagnostic criterion for posttraumatic encephalopathy. Slightly different dynamic was observed analysing the information about another enzyme involved into exchange of amino acids and peptides – GGT. At the first and second stages, a tendency of GGT serum level decrease was evidenced, which could not be significant in the patients with disease duration more than 15 years compare to the control. This data complied with the literature on GGT serum level

decrease in the patients, who suffered from severe TBI [2, 19] reflecting, in our opinion, the developing deficiency of oxidative reactions on amino acid metabolism in these patient groups and/or associated with long treatment with different painkillers and/or antiepileptic drugs.

At present, there is still no effective pharmacological treatment that stops the progression of secondary brain injury; the pathogenetic mechanisms of secondary traumatic brain injuries development are still unclear and urgent. Thereby, the further research on any other related markers and membrane-associated enzymes involved in internal redox reactions in the patients with long-term consequences of traumatic brain injury is topical.

Conclusion

Thus, in the patients with long-term consequences of mTBI, dysregulation of enzymes activity was detected that might be a marker of nervous system energy processes impairment and membranes destruction. The severity of the changes in the serum levels of membrane-associated enzymes markers correlated with the development of oxidative stress and metabolic processes dysfunction. The revealed dysregulation of enzymes activity might be caused by dystrophic cell changes, their disfunction and metabolic changes reflecting the severity of neurological symptoms.

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Conflict of Interests

The author declares no conflict of interest.

СТАН МЕТАБОЛІЧНИХ ПРОЦЕСІВ У ХВОРИХ З ВІДДАЛЕНИМИ НАСЛІДКАМИ ЛЕГКОЇ ЗАКРИТОЇ ЧЕРЕПНО-МОЗКОВОЇ ТРАВМИ

Є.В. Лекомцева

ДУ "ІНСТИТУТ НЕВРОЛОГІЇ, ПСИХІАТРІЇ ТА НАРКОЛОГІЇ НАЦІОНАЛЬНОЇ АКАДЕМІЇ МЕДИЧНИХ НАУК УКРАЇНИ", ХАРКІВ, УКРАЇНА

Вступ. *Легка закрита черепно-мозкова травма (ЗЧМТ) призводить до розвитку різноманітних порушень метаболічних процесів, що відіграють певну роль у розвитку її віддалених наслідків.*

Метою роботи *було вивчення аналізу активності деяких маркерних мембрано-зв'язаних ферментів, що приймають участь у різних окислювально-відновлювальних реакціях, метаболічних та енергетичних процесах.*

Методи. *У сорока семи хворих із віддаленими наслідками легкої ЗЧМТ та тридцяти здорових осіб були проведені обстеження та визначення активності аланінамінотрансферази, аспартатамінотрансферази, лужної фосфатази та лактатдегідрогенази (ЛД) у сироватці крові хроматографічним та колориметричним методами.*

Результати. *Дане дослідження виявило значущі зміни вмісту лужної фосфатази та ЛД, які є маркерами ушкодження клітинних мембран, важкості ацидозу, роз'єднання окислювально-відновлювальних процесів та порушення енергетичного обміну у клітинах. Середній рівень ЛД був 662,7 МО/л vs 381,9 МО/л у контролі. Виявлене збільшення рівню ЛД прямо пропорційно корелювало з прогресуванням захворювання, досягаючи найбільш значущих змін у групі хворих з тривалістю захворювання більш 15 років (mean±SD 144,6±16,3 МО/л vs 82,6±8,4 МО/л; p<0,05). Віддалені наслідки після перенесеної легкої ЗЧМТ також характеризувалися статистично значущим зниженням активності лужної фосфатази (p<0.05) та позитивною кореляційною залежністю (r=+0.48) від тривалості посттравматичного періоду, при цьому загальний рівень лужної фосфатази був 152,5±11,21 МО/л vs 212,6±9,63 МО/л у здорових (p<0,01). Ступінь вираженості змін активності мембрано-зв'язаних ферментів був пов'язаний з розвитком оксидативного стресу та дисметаболічних процесів.*

Висновки. *У хворих з віддаленими наслідками легкої ЗЧМТ було виявлено дисрегуляцію ферментативних процесів, що є маркером порушення різних видів енергетичного обміну у нервової системі та деструкції клітинних мембран.*

КЛЮЧОВІ СЛОВА: **віддалені наслідки; легка закрита черепно-мозкова травма; мембрано-зв'язані ферменти; метаболічні процеси.**

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CONNECTIVE TISSUE DISEASES: FOCUS ON MICROCIRCULATORY BED

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Background. *A microcirculatory bloodstream is a target, source and reason of the pathological process in patients with systemic connective tissue diseases.*

Objectives. *This study is focused on meta-analyses of biopsy material of skin flaps harvested from patients' fingers to identify specific morphological changes.*

Methods. *A retrospective analysis of the medical records of 39 examinees with systemic sclerosis (SSc), 45 with Systemic Lupus Erythematosus (SLE), and 45 with rheumatoid arthritis (RA) was performed. The condition of peripheral hemodynamics was examined with longitudinal rheovasography of arms and legs. Endothelin-1 (ET1) concentration was evaluated by immunoenzymatic method. We assessed other results of clinical and laboratory tests to compare them with morphological changes of the microcirculatory bed.*

Results. *Most patients involved suffered from abnormal peripheral hemodynamics. It was revealed that kidneys, lungs or heart were damaged more frequently in the patients with peripheral blood circulation disorders, which were the most significant in the patients with SSc ($p<0.05$). Disorders of peripheral blood flow were exacerbated in case of lengthening of the disease course. Concentration of ET1 was relevantly higher in the patients with peripheral blood flow disorders. Number of pathologic capillaries was the highest in the SSc patients.*

Conclusions. *In terms of integral estimation, extremely significant changes of microcirculatory bloodstream were evidenced in the patients with SSc. However, some morphometric peculiarities were revealed in the patients without peripheral blood flow disorders. Thus, normal rheovasography did not exclude any microcirculation disorders.*

KEY WORDS: **microcirculation; systemic lupus erythematosus (SLE); systemic sclerosis (SSc); rheumatoid arthritis (RA); biopsy; rheovasography**.

Introduction

Manifestations of systemic connective tissue diseases are very diverse. However, one thing they all have in common is vasculitis/vasculopathy that results in systematicity and development of permanent malfunctioning of body organs and systems. Several studies show that patients with systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and rheumatoid arthritis (RA) experience such symptoms as Raynaud syndrome, livedo reticularis, recurrent thrombophlebitis, digital vasculitis, capillaritis of palms and soles, etc. Among the manifestations listed above the most common sign is Raynaud syndrome that is characterized by cascade disruption of microcirculation: destruction of vascular endothelium, capillary basement membrane reduplication, intimal proliferation of smooth muscle cells with collagen hyperproduction and predisposition to vasoconstriction, vascular wall thickening with luminal occlusion, which is manifested by generalized clinical symptoms. Meanwhile, endothelium is a target, source and reason of the pathological process. It becomes a receptive field for binding of circulating immunoglobulins, immune complexes, a complement and attacks of sensitized T-lymphocytes. It itself produces vascular endothelial growth factor, endothelin 1, etc. Similar findings are presented in the literature [1, 2, 3, 4].

Numerous studies have been conducted in order to identify noninvasive methods of studying microcirculatory bloodstream and detect specific pathological characteristics in favour of one or another connective tissue disease to help confirm a diagnosis. Consequently, the method of nailfold videocapillaroscopy is relevant [5, 6, 7]. Moreover, the results of nailfold videocapillaroscopic examination are included into diagnostic classification by EULAR criteria of systemic sclerosis [5], indicating their high specificity and sensitivity.

For an overall assessment of capillaroscopic pattern, the following indicators are used: capillary length, intercapillary distance, loop diameter, internal diameter, capillary width,

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apex width, venous limb, arterial limb [7]. Data analysis shows typical changes, for example, SSc: giant capillaries, loss of capillaries with areas of avascularisation, ramified capillaries with pathological neoangiogenesis and severe derangement of capillary structure [5]. Nailfold capillaroscopic form changes are less symptomatic in cases of other diseases, for example in SLE. According to the literature concerning the above-mentioned pathology, they are present in 30-75% of cases. SSc (in contrast to SLE) characteristically reveals these changes that occur in 99% of cases at various stages [5, 6]. The most frequent alterations in the patients with SLE include increased tortuosity, elongated loops, and meandering bizarre loops [5]. The patients with RA are reported to have increased capillary tortuosity and prominent, clearly visible subpapillary venous plexus [5]. At the same time, according to the study of autoimmune processes, in some pathological conditions, correlation between the rate of biologically active substances and results of capillaroscopy was revealed. For example, in cases of SLE a relation between concentration of endothelin-1 (ET-1) and angioscopic characteristics of microcirculatory bloodstream (MCB) was found [8]. Meanwhile, increase in anti-endothelial antibody titre in cases of SSc was present in 22-86% of cases and combined primarily with lung damage and peripheral vascular bed [4].

A few researches describe the results of immune histochemical tests that allows establishing that CD20+, B-lymphocytes, macrophagocytes and dendritic cells, Ig G, A, M, and a complement in the form of deposits along elastic membrane of a vascular wall prevail among the cells that infiltrate a vessel wall [9].

The revealed morphological abnormalities and immunological disorders in systemic diseases of connective tissue combined with endothelial dysfunction and coagulation system disorders create favourable conditions for development of severe systemic complications.

The research is aimed at a retrospective study of morphological condition of a peripheral vascular bed in the patients with SLE, SSc, and RA to compare morphological changes with clinical and laboratory data.

Methods

Retrospective analysis of the medical records of 129 patients with rheumatic diseases was performed: 39 were diagnosed with SSc, 45 – with SLE, and 45 – with RA (all were the patients of Ternopil University Hospital, Department of Rheumatology, 2001-2004). The statistical analysis revealed a typical situation for this category of diseases – predominance of women (86.05%) of fertile age. The average duration of the disease was 8.14±0.53 years. The archive of biopsy material of skin flaps harvested from patients' fingers was reviewed that allowed morphometric analysis. The decision for biopsy was made by a rheumatologist during the treatment at the Department of Rheumatology. For histological studies, pieces of skin were fixed in 10% neutral formalin solution. Subsequent processing of the material followed by embedment in paraffin blocks was carried out by a conventional technique. The sections obtained by a sliding microtome were stained with hematoxylin-eosin. The histological specimens were studied using the optical microscope SEO SCAN and images were made with the Vision CCD Camera with a histological image display system. The condition of peripheral hemodynamics was examined by longitudinal rheovasography of forearm and legs by means of software-hardware complex of automated analysis USRH-1 ("УСРГ-01"). In the process of rheovasographic analysis the following medical parameters were estimated: the regularity of pulse waves, their appearance – upward and downward gradients, the type of a top (apex), extent of incisura, presence of additional waves, their localization on the descending part of a curve. The following measurements were analysed:

As – percussion systolic wave amplitude of a rheogram, Ohm;

RI – rheographic index, that measures a magnitude of pulse volume, in other words a systolic flow; that is a correlation of a variable and a constant dimensions of impedance of the researched area, namely, a percussion wave of a rheogram and calibration impulse magnitude, relative units (RU);

Ai – interval between the isoline and incisures on the descending part of a curve, Ohm;

DI – dicrotic index, which reflects the condition of the tone of arteries and precapillary vessels, %.

The main indicator that was used for analysis was a rheographic index that measured the magnitude of pulse volume that was a systolic flow defined by a ratio of a percussion wave of a rheogram and calibration impulse magnitude, relative units (RU).

The condition of endothelium was examined in terms of one of the main integral indicators of its functional capacity, i.e. ET1. The contents of ET1 were determined using an immuno-

enzymatic method, which was based on the principle of competitive immunoenzymatic analysis, involving a reagent kit Peninsula Laboratories Inc. (USA).

The severity of inflammation was determined by erythrocyte sedimentation rate (degree 1 – ESR <20 mm/hr, degree 2 – ESR 20-40 mm/hr, degree 3 – ESR >40 mm/hr).

All case histories contained informed consents for the research. The study was conducted in accordance with the Declaration of Helsinki. The Protocol was approved by the Committee on Bioethics of I. Horbachevsky Ternopil National Medical University.

Statistical processing of the research results was performed by parametric analysis and non-parametric analysis using the Student's t-test and Mann-Whitney U-test by means of software package Microsoft EXCEL 5.0 and Statistica 10 (StatSoft). p values lower than 0.05 were considered to be statistically significant.

Results

All cases were divided into two groups according to the state of peripheral hemodynamics, which was examined using longitudinal rheovasography of forearm and legs.

The majority of patients, the model participants of the research, had abnormal peripheral hemodynamics (group 2). The ratio of the above-mentioned patients to the patients with normal peripheral hemodynamic parameters (group 1) was 5.1:1.0. Clinical assessment of the involved patients is presented in Table 1.

Assessment of peripheral hemodynamics allowed revealing significant changes in indices of longitudinal rheovasography of forearms and legs (Table 2).

Morphometric analysis of skin biopsy was conducted in 26 model patients (Table 3, Table 4).

Biopsy results of the patients with different nosology and peripheral blood circulation disorders are presented at Figures 1, 2, and 3.

Notably, microscopic examination revealed that general architectonics of vessel walls was not damaged. Visible hyperplasia of endotheliocytes in all categories of patients was also evidenced, which may be treated as an adaptive mechanism in cases of blood circulation disorders.

The results of patients' blood tests for concentration of ET1 (Table 5) pointed to its extremely high content in the patients with peripheral blood circulation disorders in contrast to donors' indices.

Table 1. Clinical Assessment of the Patients with/without peripheral blood circulation disorders

Indicant	Patients			
	with normal peripheral hemodynamic parameters (1st group)		with abnormal peripheral hemodynamics (2nd group)	
	Absolute numbers	%	Absolute numbers	%
Age (years):				
under 20	3	2.3	4	3.1
20-44	9	6.9	53	41.1
45-59	5	3.9	38	29.5
60 and above	4	3.1	13	10.1
Males	6	4.7	12	9.3
Females	15	11.6	96	74.4
SLE	9	6.9	36	27.9
SSc	1	0.8	38	29.5
RA	11	8.5	34	26.4
Activity of the 1st degree	2	1.6	52	40.3
Activity of the 2nd degree	15	11.6	41	31.8
Activity of the 3rd degree	4	3.1	15	11.6
Duration of a disease < 1 yr	3	2.3	10	7.8
Duration of a disease 1-5 yrs	6	4.7	38	29.5
Duration of a disease 5-10 yrs	5	3.9	30	23.2
Duration of a disease >10 yrs	7	5.4	30	23.2
Kidney damage	6	4.7	28	21.7
No kidney damage	15	11.6	80	62.0
Cardiac involvement	7	5.4	37	28.7
No cardiac involvement	14	10.9	71	55.0
Lung damage	5	3.9	23	17.8
No lung damage	16	12.4	85	65.9

Table 2. Indices of peripheral blood circulation in the patients of group 2 (with peripheral hemodynamic disorders)

Indices		Forearms				Lower legs			
		As, Ohm	RI, RU	Ai, Ohm	DI, %	As, Ohm	RI, RU	Ai, Ohm	DI, %
Duration of disease	< 1 yr, n=10	0.123± 0.002*	1.23± 0.02*	0.090± 0.002*	73.41± 0.08*	0.147± 0.003*	1.47± 0.03*	0.105± 0.002	71.58± 0.08*
	>1-5 yrs, n=38	0.091± 0.002*	0.91± 0.02*	0.075± 0.002	82.24± 0.06*	0.145± 0.002*	1.45± 0.02*	0.116± 0.002*	80.24± 0.03*
	>5-10 yrs, n=30	0.087± 0.003*	0.87± 0.03*	0.074± 0.002	84.98± 0.08*	0.127± 0.003*	1.27± 0.03*	0.106± 0.003*	83.49± 0.04*
	>10 yrs, n=30	0.085± 0.002*	0.85± 0.02*	0.074± 0.001	87.18± 0.05*	0.114± 0.003*	1.14± 0.03*	0.096± 0.002	86.26± 0.04*
Disease	SLE, n=37	0.109± 0.002*	1.09± 0.02*	0.083± 0.002*	75.79± 0.05*	0.130± 0.002*	1.30± 0.02*	0.108± 0.008*	83.39± 0.04*
	SSc, n=37	0.082± 0.002*	0.84± 0.02*	0.072± 0.002	85.73± 0.08*	0.113± 0.004*	1.13± 0.04*	0.095± 0.003	84.41± 0.06*
	RA, n=34	0.089± 0.002*	0.89± 0.02*	0.070± 0.002	79.06± 0.06*	0.122± 0.003*	1.22± 0.03*	0.099± 0.003	81.11± 0.03*
Activity of the inflam-matory process	1st degree, n=52	0.095± 0.002*	0.95± 0.02*	0.080± 0.002*	84.15± 0.05*	0.121± 0.004*	1.21± 0.04*	0.099± 0.002	81.60± 0.09*
	2nd degree, n=41	0.095± 0.003*	0.95± 0.03*	0.080± 0.002*	84.17± 0.05*	0.113± 0.002*	1.13 0.02*	0.095± 0.002	84.15± 0.04*
	3rd degree, n=15	0.105± 0.005*	1.05± 0.05*	0.080± 0.004	75.52± 0.07*	0.138± 0.002*	1.13± 0.02*	0.111± 0.002*	80.44± 0.06*
Follow-up control		0.152± 0.008	1.52± 0.08	0.069± 0.005	45.43± 0.04	0.181± 0.012	1.81± 0.12	0.088± 0.008	78.53± 0.07*

*Notes. * – p<0.05 – statistically significant difference between the indices in apparently healthy people of the control group and other studied patients.*
RA – rheumatoid arthritis;
SSc – systemic sclerosis;
SLE – systemic lupus erythematosus.

Table 3. Average density of skin hemocapillaries of the patients per 1mm²

Groups	No peripheral blood circulation disorders (group1, n=11)	Peripheral blood circulation disorders (group 2, n=15)
Normal range	43.0±1.9	–
Rheumatoid arthritis, n=8	40.0±1.8 p1>0.05	37.0±1.6 p^1>0.05 p^2>0.05
Systemic sclerosis, n=8	38.0±1.7 p1>0.05	21.0±1.0 p^1<0.01 p^2<0.01
Systemic lupus erythematosus, n=10	41.0±1.9 p1>0.05	36.0±1.7 p^1<0.05 p^2>0.05

Notes:
p^1 – statistically significant difference between the normal range and average density of hemocapillaries per unit area in patients of both groups;
p^2 – statistically significant difference between the indices in patients of the groups 1 and 2.

At the same time, concentration of ET-1 in the patients without vessel disorders is not substantially different from normal range.

Discussion

Among the examinees, the detection rate of peripheral hemodynamic disorders in the

Table 4. Distribution of skin hemocapillaries of the patients over the duct width

Disease	No peripheral blood circulation disorders (group 1, n=11)			Peripheral blood circulation disorders (group 2, n=15)		
	The number of hemocapillaries and their diameter			The number of hemocapillaries and their diameter		
	8-12 μm	15-25 μm	above 25 μm	8-12 μm	15-25 μm	above 25 μm
Normal range	100	–	–	100	–	–
Rheumatoid arthritis, n=8	80.6±3.6 $p^1<0.01$	12.1±0.6	7.3±0.3	31.3±1.5 $p^1<0.01$ $p^2<0.01$	46.6±2.2	22.1±1.0
Systemic sclerosis, n=8	71.7±3.3 $p^1<0.01$	24.5±1.1	3.82±0.17	8.0±0.3 $p^1<0.01$ $p^2<0.01$	58.9±2.8	33.1±1.5
Systemic lupus erythematosus, n=10	69.2±3.3 $p^1<0.01$	29.7±1.4	1.13±0.04	7.5±0.3 $p^1<0.01$ $p^2<0.01$	43.2±2.0	49.3±2.4

Notes:
p^1 – statistically significant difference between the normal range and the number of normal-sized capillaries (8-12 μm) in the patients of both groups;
p^2 – statistically significant difference between the number of capillaries 8-12 μm in diameter in the patients of the groups1 and 2.

patients with RA was 75.6 %, which is slightly higher than the established results of statistical researches. Peripheral blood circulation disorders were diagnosed in the patients with SLE in 80 % of cases, in the patients with SSc – in 97.4 % of cases, which is consistent with literature [10, 11].

Despite nearly equal distribution of patients in every group by age, duration of a disease, activity of the inflammatory process, it has been revealed that a kidney, lung or heart are damaged more frequently in the patients with peripheral blood circulation disorders. Such results are probably associated with the development of systematicity, which is caused by peripheral blood flow and microcirculation disorders.

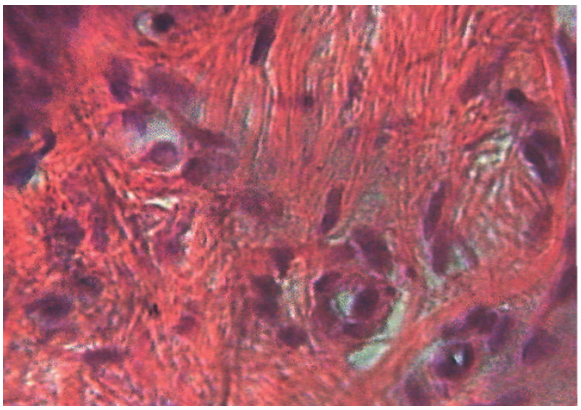

Figure 2. Biopsy material of skin flaps harvested from the finger of the patient Sh. with systemic lupus erythematosus, medical record of the in-patient patient No. 01/00920. Staining H&E. ×600

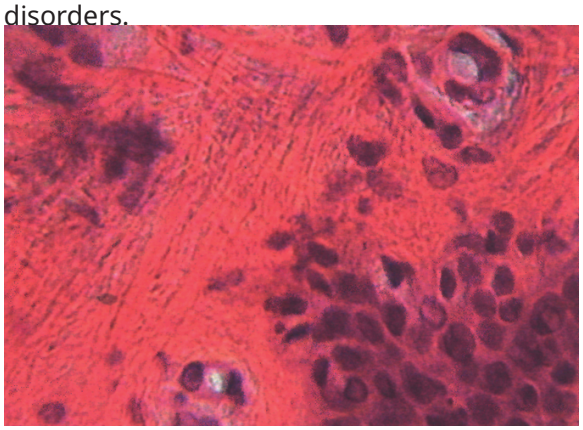

Figure 1. Biopsy material of skin flaps harvested from the finger of the patient F. with rheumatoid arthritis, medical record of the in-patient patient No. 01/10064. Staining H&E. ×600

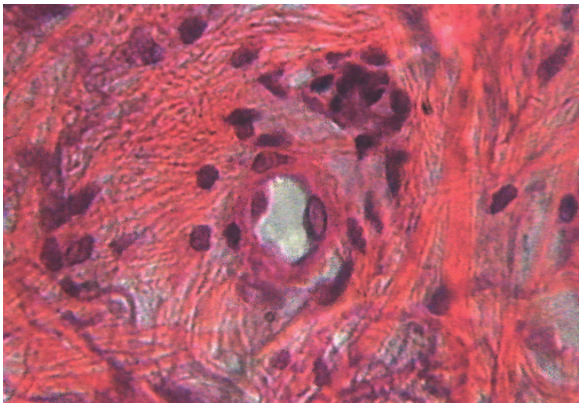

Figure 3. Biopsy material of skin flaps harvested from the finger of the patient Kh. with systemic sclerosis, medical record of the in-patient patient No. 01/07587. Staining H&E. ×600

Table 5. Concentration of ET1 in the patients with rheumatic diseases with/without peripheral blood circulation disorders

Group of patients		ET1, pkg/ml
Donors, n=10		1.8±0.7
Group 1, n=5		1.9±0.5 p^1>0.05
Group 2	SLE, n=10	19.0±1.4 p^1<0.001 p^2<0.001
	SSc, n=10	23.1±1.5 p^1<0.001 p^2<0.001
	RA, n=10	18.9±1.4 p^1<0.001 p^2<0.001

Notes:
p^1 – statistically significant difference between indices in patients and donors;
p^2 – statistically significant difference between indices in patients of the groups 1 and 2.
RA – rheumatoid arthritis; SSc – systemic sclerosis; SLE – systemic lupus erythematosus.

In the rheograms of the patients with peripheral blood circulation disorders the height of percussion wave and RI decrease is related to the increase in duration of the disease. Thus, RI in the forearms of the patients, who suffer from the disease for a period of less than 1 year, decreased by 19.07%. At the same time, the systolic blood flow in the forearms of the patients, who suffer for 10 or more years, decreased by 44.08%. Meanwhile, DI value points to hypertonia of precapillary vessels in all investigated groups, but this indice is statistically lower (p<0,001) among the patients, who suffer for a period of less than 1 year, and increases if the duration of the disease is longer.

In the patients divided by the nosological units, the lowest rates of systolic flow were observed in patients with SSc, meanwhile, RI decreased by 46.05%; the patients with RA and a deficit in RI, representing 41.45 %, were the second; consequently, the smallest changes in RI were found in the patients with SLE, whose systolic blood flow in forearms decreased by 28.29 %. The tonus of precapillary vessels, which was significantly higher in the patients with SSc (p<0.001), was changed compare to the above-mentioned data.

Analysis of rheovasography indices in terms of the degrees of inflammatory process activity (which was determined by the erythrocyte sedimentation rate) revealed no statistically significant difference. However, at the highest degree of the inflammatory process activity DI was of the smallest value and significantly different (p<0.001) from the indices at the 1st and 2nd degrees of activity.

Thus, the most significant peripheral blood circulation disorders were found in the patients with SSc (p<0.05); disorders of peripheral blood flow were exacerbated in case of lengthening of disease course. Hypertonia of peripheral vessels and decrease in systolic blood flow were reflected in flattening of a rheographic curve and rheographic index decrease. Hypertonia of arterioles and precapillary vessels was evident by high location of incisures in relation to the apex of the rheographic curve and a high DI value.

The analysis of morphometric data revealed that the average density of capillaries per unit area statistically decreased only in the patients with peripheral blood flow disorders in cases of SLE and SSc.

Along with the decrease in the number of normal-sized capillaries, dilated and megacapillaries were present. Meanwhile, their number was small in the patients without peripheral blood flow disorders and statistically significant in the patients with hemodynamic disorders. In terms of relative values, the number of pathologic capillaries was 66.7% in cases of RA, 92.0% in cases of SSc and 91.5% in cases of SLE.

The concentration of ET-1 was significantly higher in the patients with peripheral blood flow disorders. To examine the changes in concentration of ET-1 in detail, all the patients with the signs of vascular bed damage were divided into three groups by the degree of the inflammatory process activity. The revealed changes were reflected in gradual increase in concentration of ET-1 in case of a higher degree of inflammatory process activity, though there was no statistically significant difference between the indices.

According to the results of our study it has been established that microcirculatory bed changes in the patient with SSc and SLE complied with other literature regarding investigation of non-invasive methods of studying of microcirculatory bloodstream such as nailfold videocapillaroscopy [5, 6, 7]. Minimal changes have been revealed in the patients with RA [5].

Conclusions

It should be noted that peripheral hemodynamic disorders are followed by significant microcirculatory bloodstream disorders such as decrease in average density of hemocapillaries per unit area, decrease in the number of nor-

mal-sized capillaries, and increase in the number of dilated and megacapillaries. Morphometric changes are accompanied by endothelial dysfunction with a high concentration of ET-1. In terms of integral estimation extremely high level of changes is evidenced in the patients with SSc which complies with current understanding of this disease.

However, some morphometric peculiarities were revealed in the patients without peripheral blood flow disorders: in cases of adequate density of hemocapillaries per unit area, there was a decrease in the number of normal-sized capillaries and presence of dilated and megacapillaries. The above-mentioned changes were not followed by endothelial dysfunction in relation to ET1 indicator. Thus, normal rheovasography did not preclude the presence of microcirculation disorders since biopsy revealed deeper changes in microcirculatory bed.

The further research should be focused on the search for non-invasive methods of studying microcirculatory bloodstream in the patients with rheumatic diseases, which is the main cause of the development of systematicity.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Zarudna O.I. – conceptualization, data curation, formal analysis, investigation, writing – original draft and review & editing; *Venher I.K.* – conceptualization, data curation, validation, methodology; *Dovbush A.V.* – formal analysis, investigation, visualization, software.

СИСТЕМНІ ЗАХВОРЮВАННЯ СПОЛУЧНОЇ ТКАНИНИ: ФОКУС НА МІКРОЦИРКУЛЯТОРНЕ РУСЛО

О.І. Зарудна, І.К. Венгер, А.В. Довбуш

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Прояви системних захворювань є надзвичайно різноманітними. Їх об'єднує наявність васкуліту/васкулопатії, що й зумовлює системність і приводить до розвитку стійких порушень функції органів і систем. При цьому мішенню, джерелом та причиною патологічного процесу є ендотелій.*

Мета. *Провести комплексне вивчення стану мікроциркуляторного русла у хворих на системний червоний вовчак (СЧВ), системну склеродермію (ССД) та ревматоїдний артрит (РА).*

Методи. *Для досягнення мети проведено ретроспективний аналіз архівних історій хвороби 129 хворих на ревматичні захворювання, з них 39 – з верифікованим діагнозом ССД, 54 – СЧВ та 45 – РА. Переглянуто архів біоптатів шкірного клаптя пальця пацієнтів, на основі чого проведено морфометричний аналіз. Стан периферичної гемодинаміки вивчали за результатами поздовжньої реовазографії передпліч та гомілок. Вміст ЕТ - 1 визначено за імуноферментною методикою, яка ґрунтується на принципі конкурентного імуноферментного аналізу. Клінічні та лабораторні дані співставлень з результатами морфологічного дослідження біоптатів.*

Результати. *У переважної більшості пацієнтів виявлено розлади периферичної гемодинаміки. Встановлено, що ураження нирок, легень, серця частіше спостерігається у хворих з розладами периферичного кровоплину, проте найглибші порушення виявлено у хворих на системну склеродермію ($p<0,05$). Порушення периферичної гемодинаміки супроводжуються підвищенням концентрації ендотеліну-1 та поглиблюються за умови зростання тривалості хвороби. Кількість патологічних капілярів найвища у хворих на системну склеродермію.*

Висновки. *Крайній ступінь розладів мікроциркуляторного русла за інтегральною оцінкою клініколабораторних та морфометричних досліджень виявлено у пацієнтів на ССД. Однак знайдено деякі морфологічні особливості у пацієнтів без розладів периферичного кровоплину. Отже відсутність патологічних відхилень за результатами реовазографії не виключає розладів на мікроциркуляторному рівні.*

КЛЮЧОВІ СЛОВА: **мікроциркуляція; системний червоний вовчак (СЧВ), системна склеродермія (ССД), ревматоїдний артрит (РА); біопсія; реовазографія.**

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GOUT AND NONALCOHOLIC FATTY LIVER DISEASE: EFFECT OF ENTEROSORPTION'S ADDITION TO COMMON TREATMENT

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Background. *Gout is still one of the major health problems despite significant advances in treatment in recent years. It has been proved that pathogenetic mechanisms of development and progression of gout are associated with nonalcoholic fatty liver disease. Complex pathogenic treatment of patients aimed at different parts of the pathological process has recently been supplemented with the enterosorbents.*

Objective. *The aim of the research is to study the clinical features of gout with concomitant nonalcoholic fatty liver disease (NAFLD) and to evaluate the effect of carbon enterosorbent on its course.*

Methods. *123 patients were involved in the study. They were divided into 2 groups: group 1 included patients with gout without liver damage, and group 2 included patients with concomitant NAFLD. Each of these groups was divided into subgroups, in which the patients received carbon enterosorbent carboline plus basic treatment. The control group consisted of 30 healthy persons. Anamnesis, physical examination, uric acid (UA), C-reactive protein (CRP) content, erythrocyte sedimentation rate (ESR) in serum were determined. Gout activity was evaluated using the Gout Activity Score (GAS).*

Results. *Basic treatment in combination with carbon enterosorbent contributed to faster cure of intoxication, pain and joint syndromes, as well as decrease of the inflammatory process activity.*

Conclusions. *The course of gout in the patients with concomitant NAFLD is more severe. Adding of carbon granular enterosorbent carboline in the complex treatment of patients with gout with or without concomitant NAFLD in the exacerbation phase contributes to a faster cureing dynamics of clinical and laboratory manifestations of the disease.*

KEY WORDS: **gout; nonalcoholic fatty liver disease; enterosorbent; treatment.**

Introduction

Pathology of the articular apparatus is one of the most frequent and common human diseases. Salt arthropathy accounts for a large share, among which gout is the most common. Gout is a chronic progressive disease associated with impaired uric acid metabolism, which is clinically manifested by recurrent arthritis, tophi formation and internal organs damage [1].

Gout is certainly an urgent health issue and a significant social and economic burden for the country. According to the Ministry of Health of Ukraine, the number of gout patients in Ukraine has increased in recent years. Thus, in 2013, the prevalence of this disease was 167.6 per 100 thousand population, whereas in 2017 this figure reached 190.4 per 100 thousand population, and 113.9 – among the able-bodied population. The cost of treating new cases of acute gouty arthritis in the United States is estimated at $ 27.4 million annually [2, 3].

Pain and swelling of the joints during exacerbations of gout can lead to a significant deterioration and disability, which affects the patient's productivity and social activity. According to Edwards N.L. et al., a patient with gout, who is under 65 years of age, has an average of 25.1 working days a year due to the illness [4].

Uncontrolled administration of medicines for comorbid diseases, as well as malnutrition, alcohol abuse, hypodynamia, physical or psycho-emotional overload play an important role in increasing the incidence [5].

In recent years, researchers have paid particular attention to the multifaceted clinical manifestations of gout, which is not limited to damage of the musculoskeletal system and kidneys. A great number of patients suffer from obesity, disorders of lipid and carbohydrate metabolism, hypertension, which are integral parts of the metabolic syndrome [6, 7].

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According to the literature, metabolic syndrome is often aggravated by fatty liver infiltration, which can significantly affect the course of the underlying disease. Nonalcoholic fatty liver disease is the most common liver disease in the world (46% of the population, and 74% among diabetes patients) [8, 9].

Thus, the increase of gout incidence and development of concomitant pathology, which complicates its course, leads to a decrease in the effectiveness of treatment, development of complications and mortality, is an urgent issue at present that requires improved diagnosis and treatment.

Treatment of the patients with gout should be comprehensive, and include non-pharmacological and pharmacological methods. Physicians should take into account the level of uric acid (with subsequent monitoring), the form and stage of gout, radiological stage and degree of functional failure of the joints, patient's age, concomitant pathology and features of its pharmacotherapy. A key aspect of the treatment is strict diet, weight loss in obesity [10].

One of the current treatments is enterosorption, which is increasingly used in the treatment of acute and chronic diseases.

The objective of the study is to investigate clinical features of gout with concomitant nonalcoholic fatty liver disease (NAFLD) and to evaluate the effect of carbon enterosorbent on its course.

Methods

The study involved 123 patients with gout during the exacerbation period, including 118 (95.9%) men and 5 (4.1%) women. The average age was (57.73±1.01). The diagnosis of gout was based on ACR/EULAR 2015 criteria and the order of the Ministry of Health of Ukraine No. 676, dated October 12, 2006. The diagnosis of NAFLD was established according to the criteria of the Unified Clinical Protocol of Primary, Secondary (specialized) Medical Care "Nonalcoholic fatty liver disease" (Order of the Ministry of Health of Ukraine No. 826, dated November 6, 2014).

The history of the disease (duration of the disease, frequency of attacks for the last 12 months) was taken into account in clinical examination of the patients. They also underwent physical examination (determination of pain intensity by a visual analogue scale (VAS), evaluation of the number of tophi, affected joints, uric acid content (UA), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) in the blood serum.

To assess the effectiveness of therapy, the patients of both groups were monitored for complaints (pain and swelling of joints, restriction of movement in them, etc.), evaluated for joint syndrome development, dynamics of the level of UA, CRP and ESR.

Gout activity was assessed using the Gout Activity Score (GAS) suggested by Sciere C.A. et al.:

$$GAS = 0.09 \times \text{the number of attacks for the last 12 months} + 1.01 \times \sqrt{UA} + 0.34 \times \text{patient's VAS} + 0.53 \times \ln(1 + \text{number of tophi});$$

where UA – uric acid (mg/dl), VAS – visual analogue pain scale (cm) [2, 11].

All the patients were divided into two groups. Group 1 included 65 patients with gout without liver damage, group 2 – 58 patients with concomitant NAFLD. The control group consisted of 30 healthy individuals of the same age.

All the patients voluntarily agreed to participate in the study. The Patient Safety Rules and the Ethical Standards and Procedures for Research Involving Human Beings (2000) were followed during the whole study.

Statistical processing of the results was performed using Statistica 10.0 software package (StatSoft, USA) and Microsoft Office Excel 2016 statistical software package (Microsoft Corp., USA). The statistical significance of differences between the groups was evaluated using the nonparametric Wilcoxon U (Mann-Whitney) method. The differences were considered statistically significant at $p<0.05$.

Results

According to the medical history, the average duration of gout in all examined patients was (8.75±0.50) years. Clinical and laboratory characteristics of patients with gout are presented in Table. 1.

Tophi were detected in 80 (69 %) persons, in group 1 – in 36 (45 %) patients, in group 2 – 44 (55 %). It is worth noting that patients with NAFLD were predominant with multiple tophi.

When assessing the frequency of exacerbations of gout per year, a statistically significant increase in 1.5 times among the patients with concomitant NAFLD, compare to those without liver damage ($p<0.05$) should be emphasized.

The analysis of the joints involved in the pathological process in the patients with gout without liver damage proved the following joints (3.75±0.30) disorders: monoarthritis was detected in 24.6%, oligoarthritis – in 29.2%,

Table 1. Clinical and laboratory characteristics of the patients with gout

Parameter	Units	Group 1 (patients without NAFLD) (n=65)	Group 2 (patients with NAFLD) (n=58)
Age, years	M±m	59.55±1.33	55.68±1.5
Number of patients with tophi	n (%)	36 (45)	44 (55)
Number of tophi: 1-3 4-9 10 and more	n (%)	 7 (19.4) 19 (52.8) 10 (27.8)	 14 (31.8) 16 (36.4) 14 (31.8)
Number of attacks for the last 12 months:	M±m	3.03±0.23	4.67±0.19*
0 1-2 3-5 6-10 More than 10	n (%)	8 (12.3) 14 (21.5) 38 (58.5) 5 (7.7) –	– 4 (6.9) 41 (70.7) 13 (22.4) –
Number of affected joints:	M±m	3.75±0.30	5.36±0.38*
Monoarthritis oligoarthritis (2-3 joints) polyarthritis 4 and more joints	n (%)	16 (24.6) 19 (29.2) 30 (46.2)	2 (3.5) 17 (29.3) 39 (67.2)
Course: mild medium severity severe	n (%)	 17 (26) 41 (63) 7 (11)	 7 (12) 33 (57) 18 (31)
Visual analogue scale (VAS), mm	M±m	56.34±1.00	69.21±1.04*
Gout activity score (GAS)	M±m	7.53±0.28	9.63±0.35*
Uric acid, μmoL/L	M±m	459.72±11.44	621.57±13.47*
C-reactive protein, mg/l	M±m	11.35±0.71	19.58±1.62*
Erythrocyte sedimentation rate (ESR), mm/h	M±m	19.35±0.90	29.12±1.04*

Note: ***** *– statistically significant differences in the parameters between the groups of patients without and with NAFLD (p<0.05).*

polyarthritis – in 46.2%. In the patients with concomitant NAFLD, oligo- and polyarthritis were observed in the vast majority (96.5) and monoarthritis in only 3.5% of those surveyed. The average number of the affected joints in the group was (5.36±0.38).

The patients in group 2 were characterized by an increase in the number of patients with severe disease course – 18 (31%) and a decrease in the percentage with mild course – 7 patients (12%) and the average – 33 (57%). At the same time, in group 1, a severe course was diagnosed in 7 (11%), medium – in 41 (63%), mild – in 17 (26%) patients.

The intensity of pain according to VAS in the patients of group 2 was in 1.2 times higher than in group 1 (p<0.05).

The GAS scale was used to evaluate gout activity. It was found that the majority of patients had a high disease activity and, on average, in the patients with concomitant NASH it was in 1.3 times higher than in the patients without liver damage. In 36 (55.4%) patients of group 1 moderate activity (GAS 4.5-7.4) was present, and 29 (44.6%) had a high (GAS>7.4) disease activity. 45 (77.6%) patients of group 2 had high disease activity and 13 (22.4%) had moderate activity (p<0.05).

In the control group, the level of UA in the serum was (309.13±14.16) μmoL/L, CRP – (3.39±0.17) mg/l, ESR – (6.47±0.65) mm/h.

The analysis of laboratory parameters revealed that the examined group 2 experienced an increase in the level of UA in the blood in 1.4 times compare to the patients in group 1, respectively, up to (621.57±13.47) and (459.72±11.44) μmoL/L (p<0.05). The inflammatory process, by the CRP level, was more significant in 1.7 times in the patients with concomitant NAFLD than in group 1. An increase in ESR, as a criterion for the activity and severity of the inflammatory process, occurred among all patients, but mostly in the patients with concomitant liver damage (29.12±1.04) mm/h, which was 1.5 times higher than the group 1 parameters (p<0.05).

According to the treatment received by the patients, they were divided into subgroups.

Group 1 – 27 people (subgroup 1A) received treatment in accordance with the order of the Ministry of Health of Ukraine No. 676, dated October 12, 2006: hypopuricemic therapy with allopurinol according to the scheme, anti-inflammatory and analgesics (basic therapy); 38 (subgroup 1B) – additionally enterosorbent carboline 1 teaspoon 3 times a day for 10 days 2 hours before or after meals or medication. Among the patients of group 2 – 27 (subgroup 2 A) received basic therapy, 35 (subgroup 2 B), except basic treatment – carboline according to the scheme. The patients of group 2 were also treated according to the Unified Clinical Protocol of Primary, Secondary (Specialized) Medical Care "Nonalcoholic fatty liver disease" (Order of the Ministry of Health of Ukraine No. 826, dated November 6, 2014).

Pain, as one of the manifestations of articular syndrome, was the main complaint of all patients before treatment (Table 2). Swelling of the affected joint was present in 53 (81.5%) patients of group 1 and 54 (93.1%) of group 2, with redness of the skin in 45 (69.2%) and 56 (97%), respectively, local temperature rise in 50 (77%) and 54 (93.1%) individuals, body temperature rise to subfebrile numbers took place in 11 (17%) and 13 (22.4%) of both groups.

Table 2. The frequency of clinical symptoms in the patients with gout

Symptoms	Patients with gout	
	Group 1, n=65	Group 2, n=58
Joint pain and change in joint motion	65 (100 %)	58 (100 %)
Swelling	53 (81.5 %)	54 (93.1 %)
Redness	45 (69.2 %)	56 (97 %)
Local temperature rise	50 (77 %)	54 (93.1 %)
Body temperature rise	11 (17 %)	13 (22.4 %)

Clinical manifestations of the disease and improvement of personal well-being decreased in the dynamics of treatment of the patients of both groups. In the patients of group 1 a significant improvement of the general condition was observed on the 2nd-3rd day, and in the patients with concomitant liver damage – swelling, redness of the joint and the intensity of pain decreased on the 3rd-4th day of the disease, according to VAS, the body temperature was normal.

Thus, in the patients of subgroup 1A after the treatment, the intensity of pain according to VAS decreased from (55.19±1.4) mm to (18.41±3.21) mm ($p<0.001$), and from (57.16±1.4) mm to (11.66 ±2.12) mm – in the subgroup 1B ($p<0.001$).

Before the treatment, the examined of group 2 complained of severe pain in the affected joints that significantly influenced the functional activity of the patients. After baseline treatment, the pain intensity according to VAS in the subgroup 2A decreased from (69.04±1.67) mm to (30.48±4.68) mm ($p<0.001$), and in the subgroup 2B (in cases of enterosorbent administration) – from (69.31±1.34) mm to (21.20±2.82) mm ($p<0.001$).

The dynamics of UA regression in blood serum during the course of treatment in the patients of group 1 was significantly better compare to those of group 2. In cases of the baseline treatment, the level of UA gradually decreased in the subgroup 1A from (444.01±18.57) to (389.03±14.55) μmoL/L, $p<0.001$, and in the subgroup 1B with additional carboline administration – from (470.87±14.38) to (353.01±7.96) μmoL/L, $p<0.001$. Compare to the control group, this parameter was higher in 1.26 and 1.14 times, respectively. In the subgroup 2A, the level of UA decreased from (657.03±11.64) to (529.27±12.32) μmoL/L, $p<0.001$, in the subgroup 2B – from (598.29±20.14) to (473.95±7.75) μmoL/L, $p<0.001$, however, it was higher than in the control group in 1.71 and 1.53 times.

Regarding the inflammatory process activity (ESR, CRP), there was a decrease in the level of ESR ($p<0.001$) and CRP ($p<0.001$) in the dynamics of treatment in both groups. It should be noted that in the patients of subgroup 1B after enterosorbent administration the CRP concentration decreased from (12.06±1.01) to (3.51±0.37) mg/l ($p<0.001$), and ESR – from (18.47±1.14) to (7.95±0.59) mm/h ($p<0.001$), compare to the control group these values became normal ($p>0.05$). In the patients with concomitant NAFLD, in cases of the basic treatment in combination with enterosorbent there was a decrease of CRP – from (19.94±1.92) to (7.79±0.36) mg/l ($p<0.001$), ESR – from (30.51±1.37) to (11.94±0.42) mm/h ($p<0.001$), but they were in 2.3 and 1.8 times higher than in the almost healthy individuals ($p<0.001$).

Discussion

Comorbidity is still an important problem in medicine, since studying the manifestations of the combined pathology of different body systems can help uncover the mechanisms of disease formation and development of pathogenetically profound therapy [12].

At present gout is an urgent problem because its prevalence has doubled in the last decades. This disease becomes even more significant due to its comorbidity. Frequent combination of gout with concomitant pathology leads to a decrease in the effectiveness of treatment and progression of complications [13].

Currently, there is a high morbidity of NAFLD in the world, and there is a tendency to increasing incidence of this chronic liver disease in the population, development of severe complications in the disease progression that is an urgent issue of contemporary medicine. The presence of NAFLD, as a comorbid condition, enhances the inflammatory response in the body and significantly complicates the course of the underlying disease [14].

It is indisputable fact that the main link of gout pathogenesis is UA metabolism. The determination of the dynamics of UA levels in the blood in gout and concomitant NAFLD is increasingly significant as a risk factor for cardiovascular events [15].

In most such patients, prior to treatment, an increase in CRP and ESR is proved. It is established that in this pathology an inflammatory process develops, which is not only limited to local changes in the joints, but also causes a response of the whole body. The increase in CRP in the patients with gout in combination with NAFLD confirms the presence of chronic subclinical inflammation in them, and with the progression of the disease there is an increase in the level of CRP [16].

Treatment of the patients with gout should be aimed at eliminating the acute attack of arthritis and inflammatory changes in the joints, reducing the content of UA compounds in the body, eliminating extra-articular lesions associated with gout, restoring the function of the musculoskeletal system, etc. [17].

It should be noted that the use of enterosorption together with basic therapy has a positive effect on the clinical course of the disease, promotes faster regression of intoxication, pain and articular syndromes, as well as is accompanied by a decrease of inflammatory process activity in the body (CRP, ESR) and uric acid level in blood serum.

Conclusions

The course of gout in the patients with concomitant nonalcoholic fatty liver disease is more severe than in its absence. With this combination, the number of the affected joints, tophi, acute conditions increases for the period of a year. There is an increase of the intensity of pain according to visual analogue scale, as well as its activity by the Gout activity score. The inflammatory process intensifies. The inclusion of carbon granulated enterosorbent carboline in the complex treatment of the patients with gout or without concomitant nonalcoholic fatty liver disease, contributes to a faster reverse dynamic of clinical manifestations of the disease, accompanied by a more significant decrease in C-reactive protein, erythrocyte sedimentation rate and uric acid in blood serum.

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Conflict of Interests

The author declares no conflict of interest.

ПОДАГРА І НЕАЛКОГОЛЬНА ЖИРОВА ХВОРОБА ПЕЧІНКИ: ЕФЕКТ ДОДАТКОВОГО ЗАСТОСУВАННЯ ЕНТЕРОСОРБЦІЇ В ЗАГАЛЬНОМУ ЛІКУВАННІ

У.О. Мудра

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Незважаючи на те, що за останні роки досягнуто значних успіхів у лікуванні, подагра залишається однією із серйозних проблем охорони здоров'я. Особливістю сучасних хронічних захворювань є коморбідність. Доведена спільність патогенетичних механізмів у розвитку і прогресуванні подагри*

та неалкогольного стеатогепатиту. Комплексне патогентичне лікування хворих, спрямоване на різні ланки патологічного процесу, останнім часом доповнене застосуванням ентеросорбентів.

Мета дослідження. *Вивчити клінічні особливості перебігу подагри при супутньому неалкогольному стеатогепатиті (НАСГ) й оцінити вплив вуглецевого ентеросорбенту на її перебіг.*

Методи дослідження. *Обстежено 123 хворих на подагру, які перебували на стаціонарному лікуванні. Їх було поділено на 2 групи. До 1-ї групи ввійшли пацієнти з подагрою без ураження печінки, до 2-ї – хворі із супутнім НАСГ. Кожну із цих груп було розділено на підгрупи, пацієнти однієї з яких, окрім базоваго лікування, отримували вуглецевий ентеросорбент карболайн. Контрольну групу склали 30 практично здорових осіб. В клінічному обстеженні хворих враховували скарги, дані анамнезу, проводили фізикальний огляд, визначали вміст сечової кислоти (СК), С-реактивного білка (СРБ), швидкості осідання еритроцитів (ШОЕ) в сироватці крові. Активність подагри оцінювали за допомогою шкали Gout Activity Score (GAS).*

Результати. *Застосування базового лікування в поєднанні з вуглецевим ентеросорбентом сприяло швидшій регресії інтоксикаційного, больового й суглобового синдромів, а також показників активності запального процесу.*

Висновки. *Перебіг подагри у хворих з супутнім НАСГ тяжчий, ніж за його відсутності. Включення вуглецевого гранульованого ентеросорбенту карболайн в комплексне лікування хворих на подагру з супутнім НАСГ чи без нього у фазі загострення, сприяє швидшій зворотній динаміці клінічних і лабораторних проявів захворювання, супроводжується більш значним зниженням рівня СРБ, ШОЕ та СК у сироватці крові.*

КЛЮЧОВІ СЛОВА: **подагра; неалкогольний стеатогепатит; ентеросорбент; лікування.**

Відомості про автора

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MAIN INDIVIDUAL AND TYPOLOGICAL PARAMETERS OF HIGHER NERVOUS ACTIVITY IN YOUNG PEOPLE OF DIFFERENT SOMATOTYPE WITH NORMAL AND HIGH BLOOD PRESSURE

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Background. *The individual and typological features of the central nervous system are interpreted as highly genetically determined. Each somatotype is characterized by morphofunctional features of the activity of different systems, including the circulatory system.*

Objective. *The aim of the research was to study the features of the main individual and typological parameters of higher nervous activity in persons of different somatotype with normal and high blood pressure (BP).*

Methods. *In the control group of the surveyed patients the BP value corresponded to the optimal level according to the WHO classification (125 people). The second group consisted of individuals, whose systolic blood pressure exceeded 130 mmHg at the time of the study and (or) diastolic – 85 mmHg (135 people). Somatotyping technique by Carter and Heath was used. Functional mobility (FMNP) and strength of nervous processes (SNP) were determined using the Diagnost-1 program (Makarenko and Lizogub).*

Results. *In the individuals with predominance of ecto- and mesomorphic somatotype component, higher levels of major nervous processes were reported in response to strenuous processing of information, which was associated with more advanced mechanisms of information processing, its neurophysiological support. In people with endomorphic somatotype the lower levels of FMNP and SNP were clearly detected that could indicate that the speed characteristics of the nervous processes in them are at a lower level.*

Conclusions. *In normal blood pressure, the highest indicator of FMNP was found in the individuals with predominance of ecto- and mesomorphic component. In the group with high blood pressure, the indicator at the level below the average was in endomorphs. Predominance of the ectomorphic component tended to increase in the surveyed, and in the mesomorphs was at the average level. The lowest level of SNP was found in the individuals with endomorphic somatotype of both groups.*

KEY WORDS: **functional mobility of nervous processes; strength of nervous processes; blood pressure; arterial hypertension; somatotype.**

Introduction

In Ukraine, as in the rest of the world, there is an annual increase in the incidence of arterial hypertension (AH), which is particularly alarming about the so-called 'rejuvenation' of this disease. Therefore, the priority direction is prevention of hypertension and early detection of it, as it has been proven that preventive measures carried out at the initial stages of the disease give a significantly greater effect compared with the adult contingent of patients [1, 2].

Numerous epidemiological studies have shown that blood pressure (BP) is determined by the influence of both genetic factors and environmental factors. Thus, in 30% of cases, hypertension is genetically determined, and about 50% is due to factors of the environment [3].

It is established that the individual-typological features of the central nervous system are treated as highly genetically determined and responsible for the individual peculiarities of the formation, development and occurrence of electrophysiological, psychophysiological, somatic and vegetative functions [4]. The somatotype is also a genetically determined constitutional type. Considering that individuals with different types of constitution are characterized by a certain level of metabolism, which occurs due to the prevailing development of muscle, fat or bone tissue, as well as the presence of psychophysiological differences in them, it is important to study and understand the peculiarities of the course of these processes in the individuals of different somatotypes. Different

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diseases, increased physical activity change the size, contours of the body, but not somatotype [5].

The study of constitutional features is topical matter, since it provides an opportunity to implement an individual approach in assessing the psychophysiological and functional status of a person, allows predicting the peculiarities of the reaction of an organism to external influence [6]. In practical medicine, somatotype is used as an indicator of hereditary predisposition to certain diseases [7], including to arterial hypertension, which is known to be the main factor in population mortality from cardiovascular disease [8].

As each somatotype has certain morphofunctional features of the nervous, endocrine, immune systems activity, as well as the circulatory system, the structure and functions of the internal organs [7], it is especially important to isolate and study these features in the individuals of different somatotypes and with various blood pressure levels.

However, in the literature there is insufficient studies about the individual-typological features of the higher parts of the central nervous system properties in persons with different somatotypes and with various levels of blood pressure that makes the study urgent.

The aim of the research was to study the features of the main individual and typological indicators of higher nervous activity in young people aged 18-22 years old of different somatotype with high and normal blood pressure.

Methods

Our studies do not contradict the accepted bioethical norms of the Helsinki Declaration adopted by the General Assembly of the World Medical Association on Human Rights (1975-2000), the International Code of Medical Ethics and the laws of Ukraine and can be used in scientific work (decision of the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University, Protocol No. 54, dated 27.08.2019). The research was conducted in the Psychophysiological Research Laboratory of the Department of Physiology, Bioethics and Biosafety of I. Horbachevsky Ternopil National Medical University, certified by the Ministry of Health of Ukraine (Certificate No. 055/13).

Two groups of young people aged 18-22 years old were involved in the research. The control group (CG) comprised persons, whose blood pressure corresponded to the optimal level according to the WHO classification (125 persons). The second group consisted of individuals, whose systolic blood pressure exceeded 130 mmHg and (or) diastolic – 85 mmHg (135 people) at the time of study. Blood pressure was measured by Korotkov's method after resting for 5 minutes. The average result was recorded after measuring three times [9].

The somatotype of the subjects was determined using the Carter and Heath technique of somatotyping [10]. The somatotype is defined as a quantitative assessment of the present form and composition of the human body. The authors identified three constituent components: endomorphy, mesomorphy and ectomorphy. Endomorphy reflects the fat content of a human body, mesomorphy characterizes development of the skeleton and skeletal muscles, and ectomorphy demonstrates the harmony of the body and the extent of its elongation. The advantage of this scheme is the possibility of an objective assessment of each constitution component by the suggested formulas. When characterizing a somatotype, the predominant component is placed in the second place, and the next, larger, at the first.

To determine the basic individual-typological indicators of higher nervous activity – functional mobility (FMNP) and strength of nervous processes (SNP), the Diagnost-1 computer program by Makarenko and Lizogub [4], was used. As a mental load for processing of information, subject symbols (geometric figures) were used in the 'feedback mode'. When performing a test task in this mode, the signal exposure varied automatically depending on the nature of the answers: after the correct answer, the next signal exposure reduced by 20 ms, and after the wrong one, on the contrary, it extended to the same level. The range of signal exposure oscillation during operation was within 20-900 ms. Before performing the task the examined person was offered the following instruction: on presentation of the square figure as soon as possible press and release the right hand right button of the transition device, on the figure of the circle – left hand left button, and on the figure triangle none of the buttons to push (this is a brake irritant). An indicator of the maximum speed of information processing, which evaluated the property of the functional mobility of the nervous processes in this mode, according to the method, was the time of execution of the fixed load – 120 signals. The faster the subject performed the test, the higher his level of functional mobility was. The test was performed three times with 120 stimuli (geo-

metric shapes) and was evaluated for the best result. Triple testing was performed because the most optimal and stable value of the speed of information processing is achieved during the first three attempts. Based on this, Makarenko suggested the following graduation of FMNP levels in terms of task time: high – 57 and less than, higher than average – 57.1-63.5 s, average – 63.6-73.7 s, lower than average – 73.8-79.9 s, low – 87.0 and above. The determination of the strength of the nervous processes was carried out after the definition of the FMNP. The measure of the level of strength was the total number of reported and processed signals surveyed within 5 minutes. To evaluate the SNP, we used the level scale according to the revised number of signals developed by Makarenko: high level – 740 and more stimuli, higher than average – 739-691 stimuli, average – 690-630 stimuli, lower than average – 629-581 stimuli, low – 530 and and less.

The results of the study were statistically processed using a licensed statistical software package Analyst Soft Stat plus 6. The probability was estimated using the one-way ANOVA analysis based on the Tukey criterion. To compare the statistical significance of differences in the results between the groups, the method of a posterior comparison was used with an unequal number of observations. To verify the normal distribution of results, the Kolmogorov-Smirnov and Lilliefors criteria [11-13] were used.

Results

By the somatotyping method, six mixed somatotypes among all the examined ones were identified: mesomorphic endomorph, mesomorphic ectomorph, endomorphic mesomorph, ectomorphic endomorph, endomorphic ectomorph, ectomorphic mesomorph.

Functional mobility of nervous processes is characterized by the maximum rate of error-free mental load processing with the differentiation of positive and inhibitory signals [4].

According to the results of the functional mobility of the nervous processes definition in the group of subjects with normal blood pressure, high level was established in endomorphic mesomorphs and endomorphic ectomorphs. The largest number of the subjects surveyed with the level of the FMNP above the average was registered in the mesomorphic ectomorphs, and with a level below the average – in the mesomorphic endomorphs. In mesomorphic endomorphs and ectomorphic mesomorphs, the average level, which was observed in more than half of the subjects, was also prevalent, and the average level of FMNP was observed in almost all ectomorphic endomorphs. The low level of FMNP was not found in any of this group subjects (Fig. 1).

Functional mobility of nervous processes in individuals of different somatotypes with high blood pressure was lower compare to that of CG. The low level of FMNP was revealed in half of the individuals with domination of the endomorphic component of the somatotype (mesomorphic and ectomorphic endomorphs). Lower than average level was found among the endomorphic mesomorphs, meso- and ectomorphic endomorphs and amounted to about one third of the examined somatotypes. The average

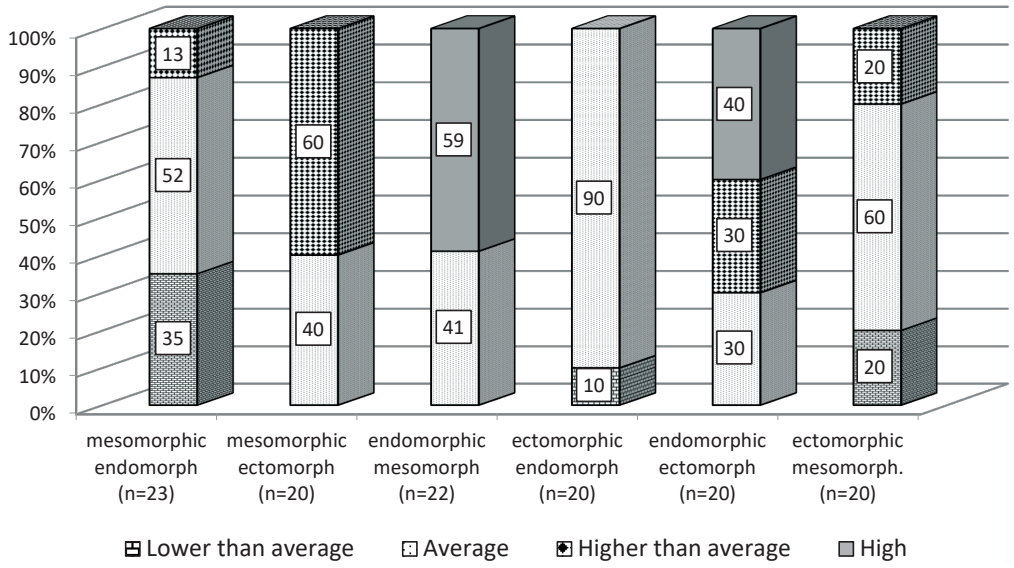

Fig. 1. Distribution of functional mobility of nerve processes levels in the persons of control group, %.

level of functional mobility predominated among endomorphic meso- and ectomorphs, and the higher than average level was observed only in individuals of ectomorphic somatotype and ectomorphic mesomorphs. The functional mobility of the nerve processes at a high level was found only in mesomorphic ectomorphs and ectomorphic mesomorphs (about 30% of the surveyed) (Fig. 2).

The strength of the nervous processes characterizes the capacity of the brain cells and manifests itself in their ability to withstand long-term concentrated excitation or the effect of short but very strong stimuli in a high tempo [4]. Therefore, a large amount of processed information indicates a higher level of SNP, and the lower one – on the reduction of brain function, and therefore the strength of the nervous processes and is characterized by a smaller amount of load performed [14]. The analysis of the attained data proved that the strength of the nervous processes in the persons of the CG at a high level was only in the small part of the endomorphic ectomorphs and ectomorphic mesomorphs, and higher than average level of SNP was observed only in them. The average SNP level was found among all somatotypes of CG, however, the largest number of individuals was among endomorphic mesomorphs and mesomorphic ectomorphs. One third of individuals from mesomorphic endomorphs and mesomorphic ectomorphs, as well as half of the representatives of ectomorphic endomorphs in the control group, were found to have the strength of the nervous processes at higher than average level. The low level of SNP was only among those with predominance of the endomorphic component in the somatotype (ecto- and mesomorphic endomorphs) (Fig. 3).

In subjects with elevated blood pressure, low levels of nervous processes were detected in all somatotypes, except for endomorphic ectomorphs. The largest number of people with this level of SNP was among ectomorphic endomorphs and was about half of the surveyed. The level below the average among the second group was registered in representatives of all somatotypes, and the largest number of subjects surveyed with this level was observed among individuals with predominance of the endomorphic component of the somatotype (meso- and ectomorphic endomorphs). The average level of nervous processes strength was recorded in half of the examined somatotypes, except for the endomorphic ectomorphs, in which the average SNP was in the largest number of individuals. The level above the mean was found only in the persons with predominance of the ecto- and mesomorphic component of the somatotype, with the highest percentages of people with this level being in endomorphic mesomorphs, and the smallest in mesomorphic ectomorphs. A high level of nervous processes strength in individuals with high blood pressure was observed only in a small amount of ectomorphic mesomorphs (Fig. 4).

According to the one-way ANOVA analysis results, the statistically significant difference between the indices of FMNP (p=0.0012) between somatotypes within the groups of the subjects under study was established. Using a

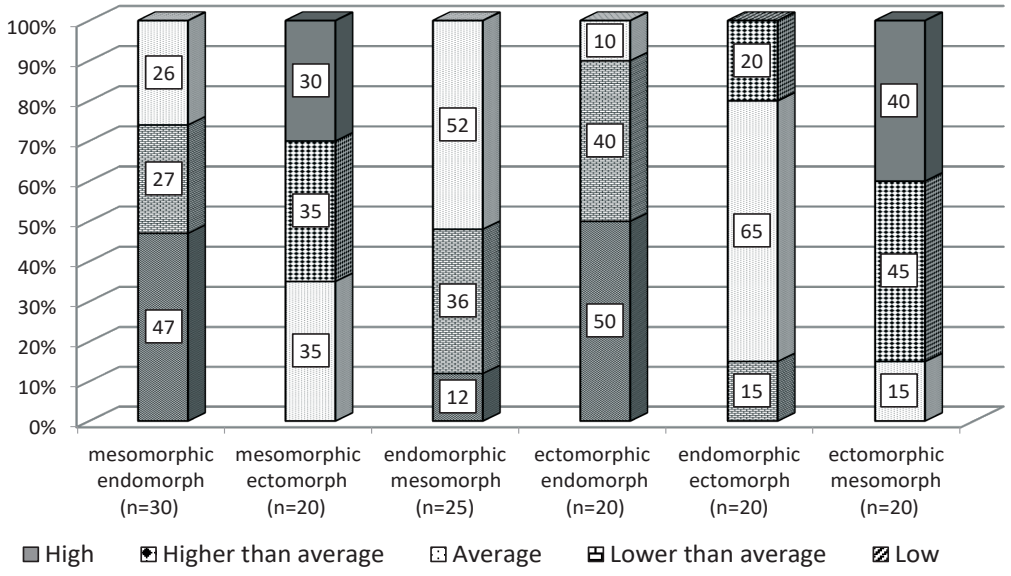

Fig. 2. Distribution of functional mobility of nerve processes levels in people with high blood pressure, %.

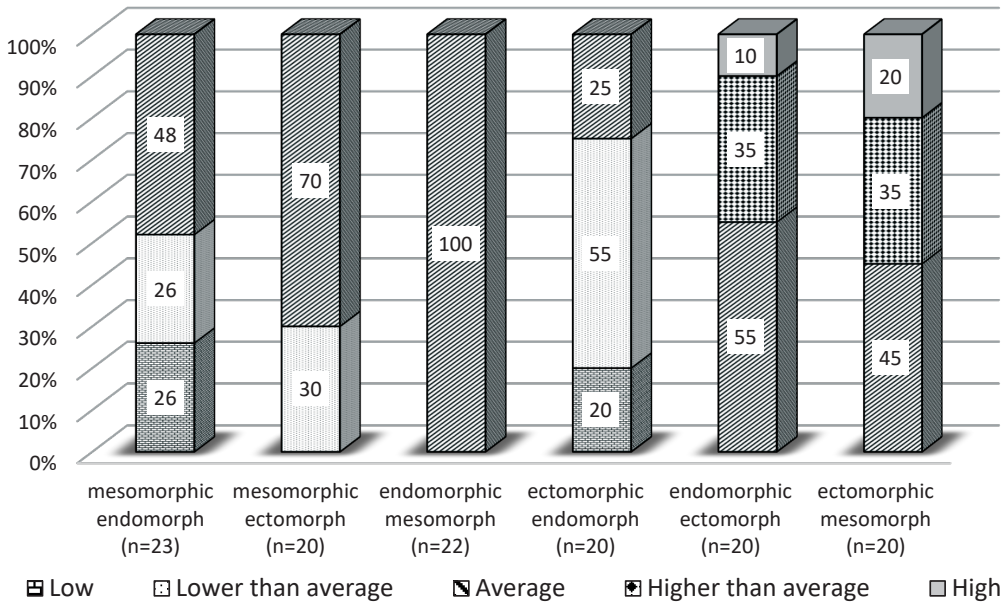

Fig. 3. Distribution of nervous processes strength levels in the persons of control group, %.

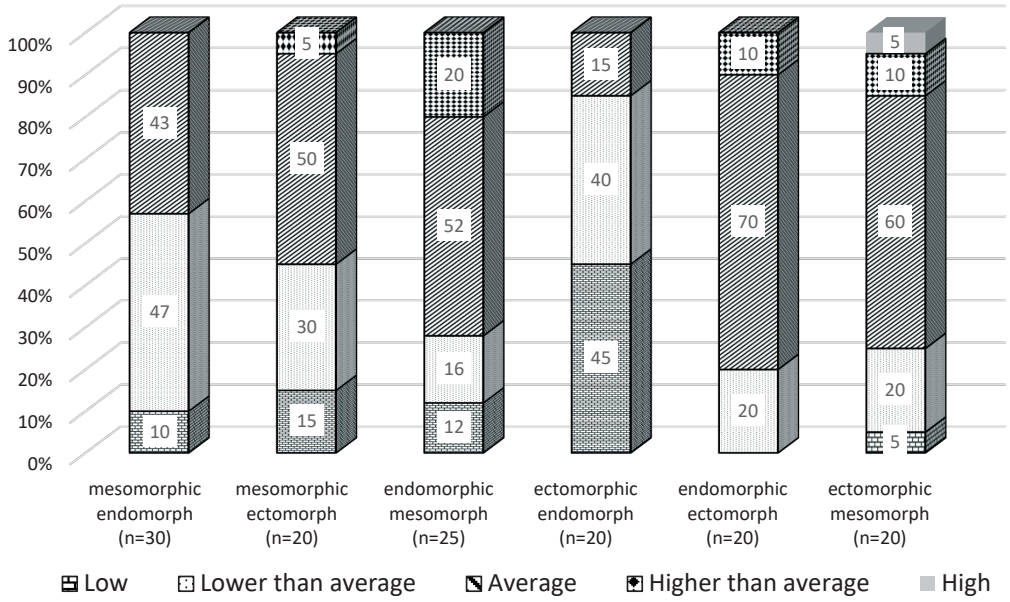

Fig. 4. Distribution of nervous processes strength levels in the subjects with high blood pressure, %.

posteriori comparison method and Tukey's criterion, the differences between somatotypes were determined by the FMNP within the groups with normal and elevated BP in the case of an unequal number of observations. In the examined CG, the difference between the indicators of functional mobility of the nervous processes was established between the mesomorphic endomorphs, in which the time of information processing was significantly longer compared to endomorphic mesomorphs (p=0.0001) and mesomorphic ectomorphs (p=0.0066). Endomorphic meso- and ectomorphs of CG showed a significant difference with ectomorphic mesomorphs (p=0.0010), in which the value of processing time was significantly lower (Table 1).

In people with high blood pressure a reliable difference of FMNP was established between the subjects with the dominated endomorphic component (meso-, ectomorphic endomorphs), in which the mean value of the task execution time was higher compared to those with predominance of the ectomorphic component of the somatotype (p=0.0006), as well as with ectomorphic mesomorphs (p=0.0002). The FMNP of mesomorphic ectomorphs and ectomorphic mesomorphs

Table 1. Parameters of FMNP in the individuals of different somatotype with normal and high blood pressure

Somatotype	Functional mobility of nervous processes			
	Subjects with normal blood pressure		Subjects with elevated blood pressure	
	n	M±m, sec	n	M±m, sec
Mesomorphic endomorphs	23	73.67±1.31	30	78.11±0.94
Mesomorphic ectomorphs	20	59.34±1.81	20	60.93±1.34
Endomorphic mesomorphs	22	69.89±0.94	25	74.14±0.97
Ectomorphic endomorphs	20	66.12±0.82	20	79.49±1.08
Endomorphic ectomorphs	20	66.63±0.61	20	68.50±1.18
Ectomorphic mesomorphs	20	59.35±0.42	20	58.33±1.02

Note. The statistical significance between the groups of the examined somatotypes and the rate of blood pressure is presented in the text.

with elevated blood pressure was significantly higher compared to those with an endomorphic component predominating in the somatotype (p=0.0006), endomorphic mesomorphs (p=0.0002), and endomorphic ectomorphs (p=0.0002). The reliable difference between FMNP in ectomorphic endomorphs of CG was established with ectomorphic endomorphs of the group of high blood pressure (p=0.0018) (Table 1).

According to the one-way ANOVA analysis results, the probable difference between the SNP indices (p=0.0036) was established among somatotypes within the groups of the subjects under study. Using a posteriori comparisons method and the Tukey's criterion, differences in groups with normal and elevated blood pressure were determined in the case of an unequal number of observations on the SNP. In the examined CG, a statistically significant difference in the strength of the nervous processes was established between mesomorphic endomorphs and endomorphic ectomorphs (p=0.0020). The number of transformed signals within 5 minutes among mesomorphic ectomorphs and endomorphic mesomorphs was significantly higher than ectomorphic endomorphs (p=0.0236). The examined ectomorphic endomorphs had a lower level of the nervous processes strength compared with those with a dominant ectomorphic component of the somatotype (p=0.0236) and with endomorphic mesomorphs (p=0.0342). The average indicator of the number of processed signals in endomorphic mesomorphs was higher and statistically different from that in the subjects with the dominant endomorphic component of somatotype (p=0.002) (Table 2).

In the group with high blood pressure, mesomorphic endomorphs had a significantly lower SNP compared with endomorphic ectomorphs (p=0.0311) and the individuals with a dominant mesomorphic component in the somatotype (p=0.0227). Endo- and ectomorphic mesomorphs had a higher level of nervous processes strength than those with domination of the endomorphic component (p=0.0427). SNP in ectomorphic endomorphs was signi-

Table 2. Parameters of SNP in the individuals of different somatotype with normal and high blood pressure

Somatotype	Strength of the nervous processes			
	Subjects with normal blood pressure		Subjects with elevated blood pressure	
	n	M±m	n	M±m
Mesomorphic endomorphs	23	618.35±9.14	30	614.03±5.05
Mesomorphic ectomorphs	20	650.40±11.70	20	631.95±9.54
Endomorphic mesomorphs	22	681.27±5.59	25	644.88±9.68
Ectomorphic endomorphs	20	614.00±6.45	20	589.70±7.41
Endomorphic ectomorphs	20	679.55±9.56	20	649.80±5.87
Ectomorphic mesomorphs	20	659.75±11.30	20	651.00±9.57

Note. The statistical significance between the groups of the examined somatotypes and the rate of blood pressure is presented in the text.

ficantly lower than those surveyed, in whom the somatotype was dominated by ectomorphic and mesomorphic components (p=0.0326) (Table 2).

Discussion

It is established that the individual-typological properties of higher nervous activity are the basis for formation of sensomotoric, autonomic and mental functions of the human body in different conditions [14]. Genetic origin of strength and functional mobility of the nervous processes parameters determines the effectiveness of any activity, especially cognitive, as well as the effectiveness of information processing of [15]. We have found that these parameters determine the functional state of the nervous system and have certain features in people of different somatotypes. In particular, in the surveyed individuals with a predominance of the ecto- and mesomorphic component of somatotype, in response to the intense work on information processing, higher levels of properties of the main nervous processes (FMNP and SNP) were recorded, which is possibly due to more advanced mechanisms for processing and evaluation of information, its neurophysiological and autonomous support.

By the nature of the data obtained, the representatives of the endomorphic somatotype clearly show presence of lower levels of the basic individual-typological characteristics of higher nervous activity that may indicate that the rapid characteristics of the course of nervous processes in the cerebral cortex are lower in them and the time of the central processing of information is longer. Regarding the state of higher nervous activity in young people with normal and high blood pressure, the level of the FMNP clearly distinguishes between persons with prevalence or presence of an endomorphic component in a somatotype. This examined functional mobility of the nervous processes is registered at a level lower than that of the same somatotypes with normal blood pressure. Such results may be explained by the fact that at increased levels of blood pressure the adaptive mechanisms increase that in turn causes changes in the work of the nervous and cardiovascular system.

Differences in the individual-typological properties of higher nervous activity in the individuals of different somatotypes can be a foundation for the successful training, obtaining labor and sports skills and using them in practical activities and can be basic in the systems of professional psychophysiological selection.

Conclusions

The analysis of the results of the level of functional mobility and the strength of the nervous processes in young people of different somatotypes showed that among the subjects with normal blood pressure, the highest index of FMNP was in people with predominance of ecto- and mesomorphic (mesomorphic ectomorphs, endomorphic ectomorphs, endomorphic mesomorphs, ectomorphic mesomorphs) component of the somatotype that could indicate a higher level of complex information processing than in the individuals of these somatotypes compared with the endomorphic component (mesomorphic endomorphs, ectomorphic endomorphs) of the somatotype. The functional mobility of the nervous processes in the group with high blood pressure was registered at lower than average level in the endomorphs, while in the surveyed with the predominance of the ectomorphic component, the index tended to increase, and the mesomorphs were on average level. The strength of the nervous processes did not differ among the individuals of different somatotypes with normal and elevated blood pressure.

The lowest level of the nervous processes strength was found among the individuals of the somatotype with domination of the endomorphic (mesomorphic endomorphs, ectomorphic endomorphs) component in both groups. This may indicate that in persons with these somatotypes, the ability to endure long-term and concentrated excitation or the action of a strong but short-term stimulus is slightly lower than that of other somatotypes without passing over to the supramental inhibition.

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Conflict of Interests

The authors declare no conflict of interest.

Authors Contributions

Vadzyuk S. N. – conceptualization, project administration, supervision, writing – review & editing; *Horban L. I.* – conceptualization, data cu ration, formal analysis, investigation, methodology, project administration, visualization, writing – original draft; *Papinko I. Ya*. – conceptualization, project administration, supervision, writing – review & editing.

ОЦІНКА ОСНОВНИХ ІНДИВІДУАЛЬНО-ТИПОЛОГІЧНИХ ПОКАЗНИКІВ ВИЩОЇ НЕРВОВОЇ ДІЯЛЬНОСТІ В МОЛОДИХ ОСІБ РІЗНОГО СОМАТОТИПУ З НОРМАЛЬНИМ ТА ПІДВИЩЕНИМ АРТЕРІАЛЬНИМ ТИСКОМ

С. Н. Вадзюк, Л. І. Горбань, І. Я.Папінко
ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Індивідуально-типологічні особливості центральної нервової системи трактуються як високо генетично детерміновані. Кожному соматотипу властиві характерні морфофункціональні особливості діяльності різних системи, в тому числі системи кровообігу, тому актуальним є вивчення їх в представників різних соматотипів та рівнем артеріального тиску (АТ).*

Мета роботи: *вивчити особливості основних індивідуально-типологічних показників вищої нервової діяльності в осіб різного соматотипу з нормальним та підвищеним АТ.*

Методи. *Відібрано контрольну групу (КГ) осіб, в яких величина АТ відповідала оптимальному рівню за класифікацією ВООЗ (125 осіб). Другу групу склали особи, у яких на момент дослідження систолічний АТ перевищував 130 мм рт. ст. і (або) діастолічний – 85 мм рт. ст. (135 осіб). Техніка соматотипування за Carter і Heath. Визначення функціональної рухливості (ФРНП) та сили нервових процесів (СНП) за допомогою програми «Діагност-1» (Макаренко та Лизогуб).*

Результати. *В осіб з переважанням екто- та мезоморфної складової соматотипу у відповідь на напружену роботу з переробки інформації зареєстровані вищі рівні основних нервових процесів, що пов'язано із більш досконалими механізмами переробки інформації, її нейрофізіологічного забезпечення. У людей ендоморфного соматотипу чітко простежуються наявність нижчих рівнів ФРНП та СНП, що може свідчити про те, що швидкісні характеристики перебігу нервових процесів в них знаходяться на нижчому рівні.*

Висновки. *В осіб з нормальним артеріальним тиском найвищим показник ФРНП був в осіб з переважанням екто- та мезоморфного компоненту. У групі з підвищеним артеріальним тиском показник на рівні нижче середнього був в ендоморфів, в обстежених із переважанням ектоморфного компоненту мав тенденцію до зростання, а в мезоморфів перебував на середньому рівні. Найнижчий рівень СНП виявлений в осіб ендоморфного соматотипу серед обох груп.*

КЛЮЧОВІ СЛОВА: **функціональна рухливість нервових процесів; сила нервових процесів; артеріальний тиск; артеріальна гіпертензія; соматотип.**

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ULTRASOUND THROMBOELASTAGRAPHY FOR THE CHOICE OF TREATMENT OF PATIENTS WITH POSTOPERATIVE VENOUS THROMBOSIS

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Background. *The frequency of venous thromboembolic complications in surgery practice is rather high. In many cases, it is the cause of a fatal pulmonary embolism. One of the actual tasks of ultrasonic diagnostics of acute venous thrombosis is the visual assessment of the substrate of the disease because it determines angiosurgical tactics and surgical prophylaxis of pulmonary embolism.*

The objective *was to prevent the development of pulmonary embolism in patients with postoperative venous thrombosis of the inferior vena cava system.*

Methods. *Vena cava system investigation and the determination of the sonoelastographic properties of the venous thrombus were carried out with the Siemens Acuson S2000 ultrasound system. The localization and prevalence of the thrombotic process were established. At the end of the topical diagnosis of a venous thrombus, the sonoelastographic properties of the thrombus were studied by determining the speed of propagation of the acoustic wave.*

Results. *The work is based on the results of examination and surgical treatment of 729 patients, of which 205 (28.12%) had operative interventions on the musculoskeletal system, 378 (51.85%) – on the abdominal organs, 146 (20.01%) – reconstructive surgery on the aorta and the main arteries of the lower extremities.*

Conclusions. *Embolodengerous thrombi are those venous thrombi of the inferior vena cava system which at ultrasonoelastography of the proximal segments of the venous thrombus are characterized by the acoustic wave propagation velocity within 2.5–2.8 m/s. The detection of embolic venous thrombosis is an indication for surgical methods prevention of pulmonary embolism*.

KEY WORDS: **pulmonary embolism; postoperative deep vein thrombosis; inferior vena cava.**

Introduction.

The frequency of venous thromboembolic complications ranges from 10 to 40% in patients with a surgical profile [1]. Postoperative venous thrombosis in 5-10% is the cause of fatal pulmonary embolism (PE) [2, 3]. One of the actual tasks of ultrasonic diagnostics of acute venous thrombosis (AVT) is a visual assessment of the substrate of the disease since the results obtained determine angiosurgical tactics of treatment and method of surgical prophylaxis of PE if it is necessary [4].

The risk of developing lower extremity deep vein thrombosis and pulmonary artery thromboembolism is higher in patients with surgical diseases. However, more than half of intra-hospital fatal episodes of pulmonary artery thromboembolism are recorded in patients with a nonsurgical profile. According to the Framingham study, pulmonary embolism accounts for 15.6% of all in-hospital mortality, with surgical patients accounting for 18% and 82% of patients with therapeutic pathology. Long-term mobility limitation of neurologic patients, inoperable cancer and hematologic diseases, complex pathology in elderly patients, and other risk factors are no less threatening predictors of venous thromboembolism than surgery.

Along with this, ultrasound examination in patients with suspected AVT can establish a correct diagnosis only in case of typical manifestations of the disease, while the frequency of diagnostic errors reaches 50% [5]. In several cases, fatal PE after the ultrasound in patients with postoperative AVT in the basin of inferior

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vena cava (IVC) [6], which encourages the search for more effective methods of diagnosis of embologenic venous thrombosis.

The **objective** of the study is to prevent the development of PE in patients with postoperative venous thrombosis of the inferior vena cava system.

Methods

The investigation of the IVC system and the determination of the sonoelastographic properties of the venous thrombus were carried out with the Siemens Acuson S2000 ultrasound system (Germany).

In the ultrasound examination of IVC system, localization and prevalence of the thrombotic process were established. At the end of the topical diagnosis of a venous thrombus, the sonoelastographic properties of the thrombus were studied by determining the speed of propagation of the acoustic wave.

At a speed of acoustic wave propagation in the range 2.5-2.6 m/s there is a high risk of embolism, while at a speed of propagation of an acoustic wave within 2.7-2.9 m/s there is a moderate risk of embolism, and at the acoustic wave propagation of 3.0 m/s and higher, the patient does not experience any risk of embolism.

All participants signed written informed consent. The trial was approved by the Ethical Committee of I. Horbachevsky Ternopil National Medical University.

Results

The work is based on the results of examination and surgical treatment of 729 patients, of which 205 (28.12%) had operative interventions on the musculoskeletal system, 378 (51.85%) – on the abdominal organs, 146 (20.01%) – reconstructive surgery on the aorta and the main arteries of the lower extremities.

According to the J. Caprini (2012) scale, 316 (43.35%) patients had a very high risk of developing thromboembolic complications, and 413 (56.64%) – a high risk. Thromboprophylaxis to patients was carried out in accordance with the provisions of the ACCP (2016).

Postoperative thrombosis in the IVC system was diagnosed in 118 (16.19%) cases. The thrombotic process in the deep vein system was diagnosed in 106 (88.89%), and varicothrombophlebitis – in 12 (10,17%) observations.

In 4 (3.77%) patients the thrombotic process in the deep veins was diagnosed at the end of the second day of the postoperative period. In 22 (20.76%) patients thrombotic process was recorded on the 3rd day after surgery, in 36 (33.96%) patients – on the 4th day, in 29 (27.36%) observations – on the 5th day, 15 (14,15%) patients – on the 6-7th day of the postoperative period.

Postoperative varicothrombophlebitis in 4 cases was diagnosed on day 4 after surgery, in 7 cases – on day 5 and in 1 case – on day 6 of the postoperative period.

At the ultrasonoelastography of the flotation segment of the ileum-femoral venous thrombus, the acoustic wave propagation velocity was 2.5-2.6 m/s (1 observation), the flotation segment of the common femoral vein was 2.5-2.6 m/s (4 observations). The proximal segment of 2.6 to 2.9 cm in length of the femoropopliteal venous thrombus was characterized by the acoustic wave propagation velocity at the level of 2.7-2.8 m/s (3 out of 45 observations).

Thrombosis of soleus and fibular sinuses with continuation into the popliteal vein was characterized by a velocity of acoustic wave propagation of 2.5–2.6 m/s (2 observations). The proximal segment with a length of 1.2-1.5 cm of the tibia-popliteal thrombus was characterized by a velocity of acoustic wave propagation within the limits of 2.7-2.8 m/s (2 out of 49 observations).

Embolic forms of postoperative deep vein thrombosis in IVC system were diagnosed on the 3rd (2 observations), 4th (7 cases) and 5th (3 observations) postoperative days.

Embolic dangerous postoperative thrombi of the deep veins of IVC system in 7 cases were found in patients after surgical interventions on the musculoskeletal system, in 4 cases – after surgery on the abdominal organs and in 1 case – after reconstructive surgery on the aorta and the main arteries of the lower limbs.

In 7 cases at ultrasonoelastography of the postoperative venous thrombus of deep vein, the propagation velocity of the acoustic wave was 2.5-2.6 m/s, which indicated a high risk of embolism of the thrombus.

In 5 cases at ultrasonogastography of a venous thrombus, the acoustic wave propagation velocity was 2.7-2.8 m/s, a moderate risk of embolism of the thrombus. In all 12 (11.32%) cases of embolic forms of deep vein thrombosis, with the aim of preventing PE, urgent surgical procedures were performed.

In one of 12 cases of postoperative varicothrombophlebitis on the 4th day after surgery, the proximal segment of the thrombotic process was localized at the level of the saphenofemoral anastomosis. The propagation velocity

of the acoustic wave of this segment of phlebothrombosis ranged from 2.7 to 2.8 m/s. Operative intervention in the form of a crossectomy and short stripping of a large saphenous vein was performed.

105 patients with postoperative venous thrombosis were prescribed anticoagulant therapy with low molecular weight heparins.

Discussion

316 (43.35%) patients underwent surgical treatment, according to the J. Caprini scale these patients had a very high risk of developing thromboembolic complications, 413 (56.65%) patients had a high risk of developing thromboembolic complications. Thromboprophylaxis at surgical interventions was carried out in accordance with the provisions of the ACCP [7]. In 118 (16.19%) cases, the development of postoperative venous thrombosis was established. Studies by several authors [8,9] state that, despite all the efforts of thromboprophylaxis measures, postoperative venous thrombosis in 5–10% is a source of PE that is fatal in 0,3–3,7% [10].

One of the actual tasks of ultrasound diagnosis at acute venous thrombosis is a visual assessment of the substrate of the disease. During the ultrasound examination, localization, the prevalence of the thrombotic process, and the shape of the apex of thrombotic masses were established [11]. The greatest danger in the development of PE is caused by two types of embolic venous thrombi: segmental floating and widespread occlusive thrombi with a floating tip. When they are detected, indications for the operative treatment of AVT become obligatory [12].

Ultrasound examination in patients with suspected AVT allows the diagnostics primarily at typical manifestations of the disease [10, 11]. Diagnostic errors may occur in the presence of fresh thrombotic masses that are not fixed to the venous wall, when the thrombotic process spreads from the veins of the tibia to the popliteal vein, with the dissemination of the thrombotic process from the soleus and peroneal sinuses into the popliteal vein.

These examples of the thrombotic process in the venous system are dangerous in terms of the development of venous thromboembolic complications [8; 12]. To establish the embolism of thrombus, a technique of determining the density of thrombotic masses is used [7]. The method does not allow to reliably estimate the results of the study since in addition to venous thrombosis the surrounding tissues are placed in the research interest zone.

A more objective and reliable method of diagnosing an embolic thrombus is the technique for determining the speed of propagation of an acoustic wave in a thrombotic mass by the sonoelastography system Siemens Acuson S2000 (Germany). At a speed of acoustic wave propagation in the range 2.5-2.6 m/s there is a high risk of embolism, at a speed within 2.7-2.9 m/s – moderate risk of embolism, at a speed of 3.0 m/s and higher there is no threat of embolism.

In 7 cases, the acoustic wave propagation velocity was established at a level of 2.5-2.6 m/s, which indicated a high risk of embolism. In 5 cases, it was determined that the acoustic wave propagation velocity was 2.7-2.8 m/s – moderate risk of embolism of the thrombus.

In all 12 (11.32%) cases of embolic forms of postoperative venous thrombosis, with the aim of preventing PE, urgent surgical procedures were performed in order to prevent PE.

A sonoelastographic method of determining the embolism of postoperative venous thrombosis was used, and when it was established, urgent surgical procedures were performed. It was possible to prevent the development of PE after surgical treatment in 729 patients with a very high (43.35 %) and high (56.64 %) risk of developing tromoboembolic complications.

Conclusions

Pulmonary embolism is one of the most common causes of death from cardiovascular disease. Clinical assessment using ultrasonoelastography helps to identify patients with clinical probability of venous thromboembolism. The results of our research demonstrated that embolodengerous thrombi of the proximal segments of the venous thrombus are characterized by the acoustic wave propagation velocity within 2.5-2.8 m/s. The detection of embolic venous thrombosis is an indication for conducting surgical methods for the prevention of pulmonary embolism.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Kostiv S.Ya. – data curation, methodology, writing – review and editing; *Khvalyboha D.V.* – formal analysis, investigation, resources, writing – original draft; *Venher I.K.* – conceptualization, supervision*; Zarudna O. I.* – validation; *Kostiv O.I.* – software.

УЛЬТРАЗВУКОВА ТРОМБОЕЛАСТОГРАФІЯ У ВИБОРІ ЛІКУВАННЯ ХВОРИХ З ПІСЛЯОПЕРАЦІЙНИМ ВЕНОЗНИМ ТРОМБОЗОМ

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ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ

Вступ. *Частота венозних тромбоемболічних ускладнень в хірургічній практиці досить висока. У багатьох випадках це причина летальної тромбоемболії легеневої артерії. Одним з актуальних завдань ультразвукової діагностики гострого венозного тромбозу є візуальна оцінка субстрату захворювання, оскільки він визначає ангіохірургічну тактику та хірургічну профілактику тромбоемболії легень.*

Метою дослідження *було запобігти розвитку тромбоемболії легеневої артерії у пацієнтів із післяопераційним венозним тромбозом системи нижньої порожнистої вени.*

Методи дослідження. *Дослідження системи порожнистої вени та визначення соноеластографічних властивостей венозного тромбу проводили за допомогою ультразвукової системи Siemens Acuson S2000. Встановлено локалізацію та поширеність тромботичного процесу. Наприкінці актуального діагнозу венозного тромбу вивчали соноеластографічні властивості тромбу шляхом визначення швидкості поширення акустичної хвилі.*

Результати й обговорення. *Робота заснована на результатах обстеження та хірургічного лікування 729 пацієнтів, з них 205 (28,12%) оперативних втручань на опорно-руховому апараті, 378 (51,85%) – на органах черевної порожнини, 146 (20,01%) – реконструктивна хірургія на аорті та основних артеріях нижніх кінцівок.*

Висновки. *Емболонебезпечні тромби – це венозні тромби системи нижньої порожнистої вени, які при ультрасоноеластографії проксимальних сегментів венозного тромбу характеризуються швидкістю поширення акустичної хвилі в межах 2,5–2,8 м / с. Виявлення емболічного венозного тромбозу є показанням до хірургічних методів профілактики тромбоемболії легеневої артерії.*

КЛЮЧОВІ СЛОВА: **тромбоемболія легеневої артерії; післяопераційний тромбоз глибоких вен; нижня порожниста вена.**

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RISK FACTORS FOR FEMALE INFERTILITY AT A TERTIARY HEALTH FACILITY IN AKURE, SOUTH-WEST NIGERIA

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Background. *The inability of couples to achieve pregnancy is a major cause of psycho-social problems in family relationship that could lead to marital disharmony.*

Objective. *The aim of this study was to find out the possible risk factors for female infertility.*

Methods. *A case-control design and a sample size of 400 (200 cases of infertility and 200 controls) were used in the study. Cases and controls were selected at random at the infertility and family planning clinic of the University of Medical Sciences Teaching Hospital Complex, Akure and were subjected to a predesigned interviewer administered questionnaire to collect the data. The cases were classified into primary and secondary infertility; binary and stepwise logistic regressions were used to generate the Odds ratio and 95% confidence interval of the possible risk factors and the level of significance was set at P<0.05.*

Results. *The mean age of the women with infertility was 28.5±5.43 years and the mean age of those in the control group was 29.1±5.62 years. Among the cases, 155 (77.5%) had secondary infertility, while 45 (22.5%) had primary infertility. Significant risk factors for female infertility included presence of fibroids, having had fibroid operation, multiple sexual partners, previous abortion, polycystic ovary syndrome (PCOS), sexually transmitted infection (STI) and post abortion sepsis.*

Conclusion. *The study showed that secondary infertility is still the most prevalent and the risk factors were multi factorial. Efforts should be intensified to reduce infertility due to preventable causes.*

KEY WORDS: **female infertility; risk factors; fibroids; polycystic ovary syndrome; sexually transmitted infection.**

Introduction

Infertility is the inability of a couple to achieve pregnancy over an average period of one year despite adequate, regular unprotected sexual intercourse [1]. WHO in 1991 estimated that between 8 and 12% of couples experienced some form of infertility during their reproductive lives, thus affecting 50 to 80 million worldwide, out of which 20-35 million couples in Africa expected to experience this problem [2]. This can be extrapolated to 3-4 million Nigeria couples suffering from infertility [3]. According to Ogunniyi in 1995, the prevalence of infertility in Sub-Saharan Africa was reported as ranging between 20-60% [4]. However, the estimate of infertile couples in Ile-Ife has been put at 19% by Okonofua in 1995 [5], although authors in previous studies in the other parts of Nigeria presented different ranging estimates.

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Causes of infertility could be due to female factors, male factors or both. Estimates show that in 35-40% of cases a man is infertile and in 35-40% of cases a woman is infertile while in 20-30% of cases, it is related to the combination of other factors [6]. Causes of female infertility include conditions that may damage the fallopian tubes, interfere with ovulation or cause hormonal imbalance [7]. These conditions include pelvic inflammatory disease resulting from sexually transmitted infections, endometriosis, polycystic ovarian syndrome, premature ovarian failure, uterine fibroids and environmental factors [7]. Other causes of infertility in females include post abortion sepsis, puerperal sepsis and age-related factors [8]. The risk factors for infertility can therefore be classified into: genital, endocrinal, developmental and general factors [9].

Infertility can be primary, if a woman has never conceived before, or it can be secondary, if a woman has at least once conceived but may or may not have carried the pregnancy to term [10]. In resource-rich countries primary infer-

tility is much more common than secondary infertility, however the reverse is the case in Sub-Saharan Africa (SSA) [11]. In some African regions, the prevalence of secondary infertility is more than 30% [12]. The high secondary infertility rate in SSA is thought to be due to sexually transmitted infections (STIs) and medical interventions under unhygienic conditions, particularly during delivery or induced abortions [13].

Infertility is a global problem affecting people around the world, which cause and importance may vary according to the geographical location and socio-economic condition; assessing risk factors for female infertility should be geographical considering the variations that could arise from different regions. The aim of this study was to evaluate the risk factors for female infertility among cases of infertility presenting to a tertiary facility in Akure, South-West Nigeria, and the type of infertility prevalent among women there. This will further help to highlight the burden of this condition and subsequently help in policy making.

Methods

Study design

The study used a matched case control design and was conducted at the infertility clinic and family planning clinic of the University of Medical Sciences Teaching Hospital Complex, Akure over a period of 2 years (July 2017- June 2019).

Sample size calculation

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_\beta + Z_{\alpha 2})^2}{(p_1 - p_2)^2}$$

where n – proportion in each group; p_2 – proportion exposed in the control group = 20%; p_1 – proportion of cases exposed; $\bar{p}$ – average proportion of cases exposed; r=1 (equal number of cases and controls), using 80% power, Z_β= 0.84 and for 5% significance level using an odd ratio of 2.0, Z_α=1.96

p_1=2.0(.20)/(.20)(2.0-1)+1=0.40/1.20=0.33,

$\bar{p}$=(.33+.20)= 0.265

$$n = 2\frac{(.256)(1-.265)(.84+1.96)^2}{(.33-.20)^2} = 181$$

Sample size = 2×181= 362, this was rounded up to 400.

A total of 200 cases and an age matched 200 controls enrolled into the study.

Consent: Informed consent was obtained from all the clients who participated in this study.

Ethical Approval: The ethical approval for the study was obtained from the Ethics Committee of the University of Medical Sciences Teaching Hospital Complex Akure, Ondo State. Protocol number 015, 12th May 2017.

Inclusion criteria: Cases were infertile women who have attended the infertility clinic at least twice and the controls were age matched women attending the family planning clinic of the same hospital that gave their consent to participate in the study.

Exclusion criteria: Women who had medications or surgery to induce pregnancy among the controls and those who did not give consent.

Sampling technique

Cases (infertile women) were selected at random from the infertility clinic of the hospital through a weekly visit till the required sample size was obtained. Controls involved fertile women, who attended the family planning clinic of the same hospital. The cases and controls were subjected to a predesigned interviewer administered questionnaire to collect socio-demographic data such as age, occupation, educational level, age at marriage; menstrual history such as age of menarche, regularity of menses, family history of infertility; relevant medical history such as diabetes mellitus, thyroid diseases, hypertension were also assessed; surgical history such as abdominal/pelvic surgeries were obtained. Information on probable gynaecological conditions that could cause infertility such as history of endometriosis, polycystic ovarian syndrome, presence of fibroids, sexually transmitted infections, genital infection following childbirth and previous abortion were obtained. The cases were classified into primary or secondary infertility.

Statistical Analysis

The collected data were analysed using the computer package SPSS version 23. Descriptive tables were generated and binary logistic regression was used to generate the Odds ratio and the 95% confidence interval of the possible risk factors for infertility. These factors were further subjected to analysis using a stepwise logistic regression to identify the main predictors of female infertility. The statistical significance was set at p<0.05.

Results

During the study, 200 cases of female infertility were registered and 200 age-matched controls. The mean age of the women with infertility was 28.5±5.43 years old and the mean age of those in the control group was 29.1±5.62 years old. Among the cases, 155 (77.5%) pa-

tients suffered from secondary infertility, while 45 (22.5%) had primary infertility. The mean ages of those with secondary and primary infertility were 28.7±4.4 years old and 29.1±3.4 years old respectively. As presented in Table 1, 96 (61.9%) cases of secondary infertility and 70 (45.2%) of their control were traders, the risk of secondary infertility was statistically significantly higher among the traders compare to their controls [OR=0.17, 95% CI=0.06-0.48, p=0.001]. Meanwhile, there was no statistically significant difference between the occupation of those with primary infertility and their control. The majority of the subjects practiced Christian religion, were married and had tertiary level of education with no statistically significant difference (p>0.05) in both groups.

Table 2 showed the distribution of female infertility and the controls according to their gynaecological history.

Most of the women in both groups were already married at the age of over 18 years old. Among those with secondary infertility, 94 (60.6%) had no living children but had abortions/ miscarriages in the past compare to their controls, where 92 (59.4%) already had ≥3 children, this was statistically significant, p=0.000. Similarly, the 45 (100%) of those with primary infertility have never been pregnant compare to their controls, where 31 (68.9%) had ≥3 living children. This was also statistically significant, p=0.000. Among those with secondary infertility, 31 (20.0%) had more than one sexual partner compare to 4 (2.6%) of the control, while 7 (15.6%) of those with primary infertility and 0 (0%) of the control had more than one sexual partner; this was also statistically significant, P=0.000. Among the cases, 91 (58.7%) of those with secondary infertility started their menses after the age of 15 years compare to 88 (56.8%) of their control, delayed menses was not a significant risk factor for infertility [OR=0.92, CI=0.59-1.45, p=0.730]. Only 10 (6.5%) of the cases of secondary infertility and 3 (6.7%) of their control reported family history of infertility, this was not a significant risk factor for infertility [OR=0.89, CI=0.35-2.26, p=0.813].

The analysis of cases of female infertility and the control according to the medical or surgical history showed that none of the subjects have ever had diabetes, thyroid disease or tuberculosis. Though, 10 (6.5%) of the cases of secondary infertility and 7 (4.5%) of their control reported to have had hypertension while 3 (6.7%) of the cases of primary infertility and none of their control reported to

Table 1. Socio-demographic characteristics of cases of female infertility and control subjects

Demographic factors	2° infertility cases (n=155) No (%)	Control (n=155) No (%)	OR (95% CI)	P value	1° infertility cases (n=45) No (%)	Control (n=45) No (%)	OR (95% CI)	P value
Age			-	0.203			0.34	0.229
15-24	0(0.0)	3(1.9)			2(4.4)	1(2.2)	(0.06-1.96)	
25-34	78(50.3)	73(47.1)			26(57.8)	19(42.2)	0.49	
≥35	77(49.7)	79(51.0)			17(37.8)	25(55.6)	(0.27-0.91)	
Occupation				0.001				0.177
Civil/public servants	54(34.8)	64(41.3)	0.28 (0.10-0.79)		15(33.3)	22(48.9)	5.86 (0.59-57.78)	
Trading/ business	96(61.9)	70(45.2)	0.17 (0.06-0.48)		26(57.8)	22(48.9)	3.38 (0.35-32.55)	
Housewives/ applicants®	5(3.2)	21(13.5)			4(8.9)	1(2.2)		
Religion				0.791				0.306
Christian	148(95.5)	147(94.8)	0.86		44(97.8)	42(93.3)	0.32	
Islam	7(4.5)	8(5.2)	(0.31-2.45)		1(2.2)	3(6.7)	(0.06-1.62)	
Marital status				0.314			–	0.153
Single	3(1.9)	1(0.6)	0.3 3		0(0.0)	2(4.4)		
Married	152(98.1)	154(99.4)	(0.03-3.19)		45(100)	43(95.6)		
Educational level			0.47 (0.18-1.23)	0.173			0.67 (1.10-4.28)	0.887
Primary	13(8.4)	7(4.5)	0.72		3(6.7)	2(4.4)	1.08	
Secondary	46(29.7)	38(24.5)	(0.43-1.20)		12(26.7)	13(28.9)	(0.42-2.75)	
Tertiary ®	96(61.9)	110(71.0)	Reference		30(66.7)	30(66.7)	Reference	

® = *reference category*

Table 2. Distribution of cases of female infertility and controls according to their gynaecological history

Factors	2° infertility cases (n=155) No (%)	Control (n=155) No (%)	OR (95%CI)	P value	1° infertility cases (n=45) No (%)	Control (n=45) No (%)	OR (95%CI)	P value
Age at marriage			–	0.132			–	0.360
<18	2(1.3)	0(0.0)			0(0.0)	1(2.2)		
≥18	151(97.4)	155(100)			45(100)	43(95.6)		
None	2(1.3)	0(0.0)			0(0.0)	1(2.2)		
Parity			–	0.000			–	0.000
1	46(29.7)	20(12.9)			0(0.0)	2(4.4)		
2	13(8.4)	43(27.7)			0(0.0)	12(26.7)		
≥3	2(1.3)	92(59.4)			0(0.0)	31(68.9)		
None	94(60.6)	0(0.0)			45(100)	0(0.0)		
Age at 1st menses			0.92 (0.59-1.45)	0.730			0.58 (0.32-1.05)	0.203
<15®	64(41.3)	67(43.2)			23(51.1)	17(37.8)		
≥15	91(58.7)	88(56.8)			22(48.9)	28(62.2)		
Family history of infertility			0.89 (0.35-2.26)	0.813		0(0.0) 45(100)	–	0.078
Yes	10(6.5)	9(5.8)			3(6.7)			
No	145(93.5)	146(94.2)			42(93.3)			
No sexual partner			9.43 (3.24-27.46)	0.000			–	0.006
1	124(80.0)	151(97.4)			38(84.4)	45(100.0)		
>1	31(20.0)	4(2.6)			7(15.6)	0(0.0)		

have had hypertension, this was not statistically significant [OR=0.69, CI=0.25-1.85, P=0.454]. However, 15 (9.7%) of secondary infertility cases have had fibroid operation compare with 1 (0.6%) of the control and the risk of secondary infertility was significantly higher than the control [OR=0.06, 95% CI=0.01-0.47, P=0.007]. Also, 5 (11.1%) of those with primary infertility and none of the control have had fibroid operation, the risk of primary infertility was significantly higher than the control, P=0.021 (Table 3).

Table 3. Distribution of cases of female infertility with controls according to their medical/surgical history

Medical conditions	2° infertility cases (n=155) No (%)	Control (n=155) No (%)	OR (95%CI)	P value	1° infertility cases (n=45) No (%)	Control (n=45) No (%)	OR (95%CI)	P value
Diabetes			–	–			–	–
Yes	0(0.0)	0(0.0)			0(0.0)	0(0.0)		
No	155(100)	155(100)			45(100)	45(100)		
Hypertension			0.69 (0.25-1.85)	0.454			–	0.078
Yes	10(6.5)	7(4.5)			3(6.7)	0(0.0)		
No	145(93.5)	148(95.5)			42(93.3)	45(100)		
Thyroid			–	–			–	–
Yes	0(0.0)	0(0.0)			0(0.0)	0(0.0)		
No	155(100)	155(100)			45(100)	45(100)		
Tuberculosis			–	–			–	–
Yes	–	–			–	–		
No	155(100)	155(100)			45(100)	45(100)		
Fibroid operation			0.06 (0.01-0.47)	0.000			–	0.021
Yes	15(9.7)	1(0.6)			5(11.1)	0(0.0)		
No	140(90.3)	154(99.4)			40(88.9)	45(100)		
Other operation			–	0.317			–	–
Yes	1(0.6)	0(0.0)			0(0.0)	0(0.0)		
No	154(99.4)	155(100)			45(100)	45(100)		

Table 4 presents the analysis of cases of infertility and the control according to their gynaecological conditions. The gynaecological conditions in the subjects included fibroids, which was present in 46 (29.7%) cases of secondary infertility compare to 11 (7.1%) of their control, the risk of secondary infertility among them was significantly higher than their control [OR=0.18, 95% CI=0.09-0.37, P=0.00].

Similarly, among the cases of primary infertility, 15 (33.3%) had fibroids compare to only 1 (2.2%) of their control, the risk of primary infertility was significantly higher than the control [OR=0.07, 95%CI=0.008-0.64, P=0.00]. Also, among the cases of secondary infertility, 50 (32.3%) had endometriosis, 35 (22.6%) of their control had similar condition, while in the cases of primary infertility 15 (33.3%) had endometriosis and 10 (22.2%) had the condition, there was no statistically significant difference in both groups at p>0.05. Among the 2 groups, 114 (73.5%) of those with secondary infertility had previous abortion compare to 53 (34.2%) of their control while none was reported in the cases of primary infertility. The risk of secondary infertility was significantly higher than the control [OR=0.19, 95% CI=0.12-0.30, p=0.000]. Post abortion sepsis was reported in 30 (19.4%) of cases of secondary infertility and only 10 (6.5%) of the control with a statistically significant higher risk among the cases [OR=0.29, 95% CI=0.14-0.61, p=0.001]. Another gynaecological condition prevalent was polycystic ovary syndrome (PCOS), which was reported in 53 (34.2%) cases of secondary infertility and 7 (4.5%) of their control, the risk of PCOS among those with secondary infertility was significantly higher than their control, [OR=0.09, 95% CI=0.04-0.21, p=0.000] while in the cases of primary infertility, it was reported in 18 (40.0%) and 2 (4.4%) of their control and the risk of PCOS was also significantly higher than the control [OR=0.01, 95% CI=0.01-0.52, p=0.000]. Sexually transmitted infection was reported in 47 (30.3%) cases of secondary infertility and 18 (11.6%) of their control with a significantly higher risk of this condition among these cases [OR=0.30, 95% CI=0.17-0.55, p=0.000), while

Table 4. Analysis of cases of female infertility and the controls according to their gynaecological conditions

Gynaecological conditions	2° infertility cases (n=155) No (%)	Control (n=155) No (%)	OR (95%CI)	P value	1° infertility cases (n=45) No (%)	Control (n=45) No (%)	OR (95%CI)	P value
Endometriosis				0.056			0.79	0.239
Yes	50(32.3)	35(22.6)	0.61		15(33.3)	10(22.2)	(0.17-3.69)	
No	105(67.7)	120(77.4)	(0.37-1.02)		30(66.7)	35(77.8)		
Fibroid diagnosis			0.18	0.000			0.07	0.000
Yes	46(29.7)	11(7.1)	(0.09-0.37)		15(33.3)	1(2.2)	(0.008-0.64)	
No	109(70.3)	144(92.9)			30(66.7)	44(97.8)		
Previous abortion			0.19	0.000			–	0.000
Yes	114(73.5)	53(34.2)	(0.12-0.30)		0(0.0)	16(35.6)		
No	41(26.5)	102(65.8)			45(100)	29(64.4)		
PCOS				0.000				0.000
Yes	53(34.2)	7(4.5)	0.09		18(40.0)	2(4.4)	0.01	
No	102(65.8)	148(95.5)	(0.04-0.21)		27(60.0)	43(95.6)	(0.01-0.52)	
Menstrual cycle			0.55	0.184			0.83	0.215
Regular	141(91.0)	147(94.8)	(0.22-1.35)		37(82.2)	41(91.1)	(0.10-6.71)	
Irregular	14(9.0)	8(5.2)			8(17.8)	4(8.9)		
Genital infection			1.26	0.627			–	0.061
Yes	8(5.2)	10(6.5)	(0.48-3.30)		0(0.0)	4(8.9)		
No	147(94.8)	145(93.5)			45(94.8)	41(91.1)		
STI				0.000				0.764
Yes	47(30.3)	18(11.6)	0.30		6(13.3)	7(15.6)	1.11	
No	108(69.7)	137(88.4)	(0.17-0.55)		39(86.7)	38(84.4)	(0.21-5.89)	
Post abortion sepsis			0.29	0.001			0.71	0.078
Yes	30(19.4)	10(6.5)	(0.14-0.61)		0(0.0)	3(6.7)	(0.0-0.0)	
No	125(80.6)	145(93.5)			45(100)	42(93.3)		

among the cases of primary infertility 6 (13.3%) and 7 (15.6%) of their control reported to have had this condition, this was not statistically significant, p>0.05. Among the cases of secondary infertility, 8 (5.2%) and 10 6.5%) of the control had genital infection, there was no statistically significant difference in both groups. Menstrual irregularity was found in 14 (9.0%) cases of infertility compare to 8 (5.2%) of their control and 8 (17.8%) of primary infertility compare to 4 (8.9%) of the control, this was not statistically significant.

Table 5 showed various predictors of secondary and primary infertility using stepwise logistic regression analysis of the various independent risk factors for infertility.

Table 5. Stepwise logistic regression to identify predictors of female infertility

Independent variable	B	S.E. (Standard Error)	Test	P. value	OR	95% CI
Model (1): For Secondary infertility						
Fibroid diagnosis	1.113	0.426	6.838	0.009	3.04	1.32-7.01
Fibroid operation	1.621	1.151	1.984	0.159	5.06	0.53-48.21
PCOS	2.213	0.45	24.174	0.00	9.14	3.78-22.08
STI	0.746	0.359	4.325	0.038	2.11	1.04-4.26
Post abortion sepsis	-0.271	0.473	0.329	0.57	0.76	0.30-1.93
Previous abortion	1.544	0.294	27.564	0.000	4.68	2.63-8.33
Constant	-12.393	2.50	24.487			
Model (2):For Primary infertility						
Fibroid diagnosis	2.508	1.118	5.034	0.025	12.28	1.37-109.86
PCOS	2.884	1.13	6.489	0.01	17.88	1.94-164.51
STI	-0.052	0.84	0.004	0.951	0.949	0.18-4.94
Constant	-12.393	2.50	24.487	0.000		

In Model 1, among the various significant factors detected by the binary logistic regression analysis for secondary infertility only the presence of fibroids [OR=3.04, 95% CI=1.32-7.01, p=0.009], polycystic ovary syndrome [OR=9.14, 95% CI=3.78-22.08, P=0.00], sexually transmitted infection [OR=2.11, 95% CI=1.04-4.26, p=0.038] and previous abortion [OR=4.68, 95% CI=2.63-8.33, p=0.000] were predictors of secondary infertility. In Model 2, the presence of fibroids [OR=12.28, 95% CI=1.37-109.86, p=0.025] and polycystic ovary syndrome [OR=17.88, 95% CI=1.94-164.51, p=0.01] were the predictors of primary infertility.

Discussion

The study showed that secondary infertility was commoner among the women (77.5%) than primary infertility (22.5%), which is in keeping with studies carried out in other parts of Nigeria [11,12,13]. It further highlights the burden of secondary infertility which is more prevalent in Sub-Saharan African countries compared to the Western world [14].The demographic characteristics of the subjects showed that most of the women were already married and possibly in a sexual relationship to enable one ascertain if there are issues concerning their fertility. Other factors such as the woman's age, religion, educational level had no impact on fertility as against a similar study which reported a decrease in a woman's fertility with increasing age [15]. These factors therefore were not risk factors for infertility among our women.

Menstrual irregularity was seen in some of the women with secondary infertility though not statistically significant and therefore suggest that this has no strong effect as a risk factor for infertility among our women. However, having multiple sexual partners was a significant risk factor for infertility among the women, which further confirmed the increase in sexually transmitted infection which was also reported among the women with secondary infertility. Sexually transmitted infections are transmitted through sexual activity with an infected partner and a major cause of secondary infertility in Sub-Saharan Africa [6].

This study also highlights gynaecological conditions such as the presence of fibroids and previous abdominal surgeries for fibroids as risk factors for female infertility which is in keeping with another study [16]. Large fibroids may cause infertility by impairing the uterine lining, blocking the fallopian tube, distorting the shape of the uterine cavity or altering the

position of the cervix. Also, following pelvic surgery, postsurgical or post infective uterine or abdominal adhesions that result may restrict the movement of ovaries and fallopian tubes and cause infertility.

Previous abortion and post abortion sepsis were significant risk factors for secondary infertility in this study. This finding has also been reported in another study in Nigeria where induced abortion and post abortion sepsis were found to affect future fertility [17]. This may be as a result of repeated injuries to the uterine lining from multiple dilatation and curettage which can cause adhesions within the uterus thus leading to secondary amenorrhea and infertility. However, genital infection did not have any significant effect on the cause of infertility, this could have been due to increase access to health care and the presence of skilled birth attendants during delivery.

This study showed ovarian dysfunction as a result of polycystic ovary syndrome to be a significant cause of both primary and secondary infertility among the women, similar studies have shown that nearly 10% of infertile women are diagnosed with reduced ovarian dysfunction and polycystic ovary syndrome implicated as a common cause of ovulation disorder in women of childbearing age [18,19,20].

In this study the main predictors of female infertility were the presence of fibroids, polycystic ovary syndrome, sexually transmitted infections and previously induced abortions for unwanted pregnancies. These findings have further revealed the great impact of reproductive infections and hormonal imbalance as major causes of female infertility.

Conclusion

Female infertility is still a major public health issue and its cause could be multi factorial. Secondary infertility remains the most prevalent type in the region mostly due to tubal damage as a result of increase in sexually transmitted infections and previous induced abortions. Though, hormonal cause such as polycystic ovary syndrome may not be under our control, reproductive infections from sexual activity could be curtailed by preventing unsafe sex and prompt treatment of diseases resulting from sexually transmitted infections. Efforts should be intensified to prevent unsafe abortions which could lead to infertility in the future.

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Conflict of Interests

The author declares no conflict of interest.

ФАКТОРИ РИЗИКУ РОЗВИТКУ ЖІНОЧОГО БЕЗПЛІДДЯ НА ТРЕТИННОМУ РІВНІ НАДАННЯ МЕДИЧНОЇ ДОПОМОГИ У АКУРЕ, ПІВДЕННО-ЗАХІДНА НІГЕРІЯ

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Вступ. *Неможливість пари народити спільних дітей є однією з головних психо-соціальних проблем, що призводить до дисгармонії подружніх стосунків.*

Мета дослідження *встановити можливі фактори ризику розвитку жіночої неплідності.*

Методи. *У дослідження було залучено 400 жінок (200 – з неплідністю та 200 осіб склали групу контролю). Жінок було обрано методом випадкової вибірки у клініці планування сім'ї при Навчально-лікувальному комплексі Університету медичних наук, Акуре. Усі досліджувані заповнювали спеціально розроблений опитувальник. Усі випадки було класифіковано на первинне та вторинне неплідддя. Застосовано статистичний регресійний аналіз: бінарну та непряму логістичну регресію, рівень значущості p<0.05.*

Результати. *Середній вік жінок з неплідністю склав 28,5±5,43 роки, контрольної групи – 29,1±5,62 роки. У 155 (77,5%) випадках мала місце вторинна неплідність, і лише у 45 (22,5%) – первинне безпліддя. Статистично значущими факторами ризику були наявність фібротичних змін, оперативні втручання з їх приводу, кілька сексуальних партнерів, попередні аборти, синдром полікистозних яйників, хвороби, що передаються статевим шляхом та постабортний сепсис.*

Висновки. *Дослідження показало, що вторинне безпліддя все ще переважає у структурі захворюваності, фактори ризику – мульфакторіальні. Зусилля, спрямовані на зменшення розвитку неплідця через фактори, які можна попередити, повинні бути посилені.*

KEY WORDS: **жіноче безпліддя; фактори ризику; фіброз; синдром полікистозних яйників; хвороби, що передаються статевим шляхом.**

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PRIMARY EPISODE OF BIPOLAR AFFECTIVE DISORDER

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Background. *Bipolar affective disorder (BAD) is a topical issue of contemporary psychiatry. The features of the primary episode (PE) of the disease are extremely important for prognosis, treatment and rehabilitation measures of BAD. Individual psychological features of the patients with PE of BAD are still unexplored that complicates development of new methods of prediction, treatment and prevention of BAD.*

Objective. *The aim of the study was to investigate individual psychological features of the patients with a primary episode of bipolar affective disorder, taking into account the gender factor and clinical variant of the BAD debut.*

Methods. *153 patients (65 men and 88 women) with a primary episode of bipolar affective disorder were examined. The patients were divided into three groups according to the clinical variant of the course of PE of BAD: depressive variant, manic variant and mixed variant. The examination was carried out using the Standardized multifactor method of personality research (SMMPR). Statistical processing of the data was performed using the non-parametric Mann-Whitney test.*

Results. *The most significant differences in the quantitative indicators of SMMPR were found when comparing depressive and manic, as well as depressive and mixed variants of PE of BAD, and lesser – when comparing manic and mixed variants. Most of all, these differences were expressed in terms of pessimism, impulsiveness, individualism and optimism.*

Conclusions. *Some peculiar features of male and female patients with depressive, manic and mixed variants of PE of BAD promoting to search for new methods of prediction, treatment and prevention of BAD have been defined.*

KEY WORDS: **bipolar affective disorder; individual-psychological features; depressive, manic and mixed variants.**

Introduction

Bipolar affective disorder (BAD) is one of the most topical matters of contemporary psychiatric science and practice. Its medical and social significance is associated with its relatively high prevalence: from 0.6% to 1.0% [1], relative stability over a long period of time [2, 3] and the presence of serious lifelong problems [4], i.e. severe affective disorders [5], cognitive disorders [6] and high mortality rates [7]. The features of the primary episode of the disease are extremely important for prognosis, treatment and rehabilitation measures of BAD; however, the initial characteristics of the disease are still poorly defined and the low prognostic value of the existing predictors requires improvement of prodromal identification means [8]. In many cases several years pass from the first manifestations of the disease to the diagnosis of BAD, despite the extreme importance of PE for prediction of the disorder severity, its functional consequences and therapeutic perspectives [9, 10]. In recent years, much attention was paid to the study of individual and psychological characteristics of the patients with BAD; these features are important predictors of the disease clinical course, treatment and rehabilitation perspectives [11]. It is established that bipolar disorder is associated with low self-esteem, paranoia and obsession [12]. At the same time, the individual-psychological features of the patients with PE of BAD are unexplored that complicates development of new methods of prediction, treatment and prevention of BAD.

The objective is to investigate the individual psychological features of the patients with a primary episode of bipolar affective disorder, taking into account gender factor and clinical variant of the BAD debut.

Methods

The study was performed in a continuous manner by examining all patients with fitting diagnoses, who sought medical care within a

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specified period. 153 patients (65 men and 88 women) with a primary episode of bipolar affective disorder treated at Ternopil Regional Psychoneurological Hospital in 2011–2016 were examined; according to biomedical ethics the patients' informed consent were received. The patients with other diagnoses were excluded from the study. The examination was performed using the Standardized multifactor method of personality research (SMMPR) [13].

Statistical data was processed using Microsoft Excel 2013 and Statistica 13 software packages. The statistical analysis included assessment of traits distribution for quantitative variables using the Shapiro-Wilk's W test, Kolmogorov-Smirnov test and Lilliefors test. The Shapiro-Wilk's W test was a reference. The non-parametric Mann-Whitney U test was used to analyze the differences between the groups taking into account the nature of the distribution (other than normal). The statistical significance of differences over 95.0% ($p<0.05$) was acceptable.

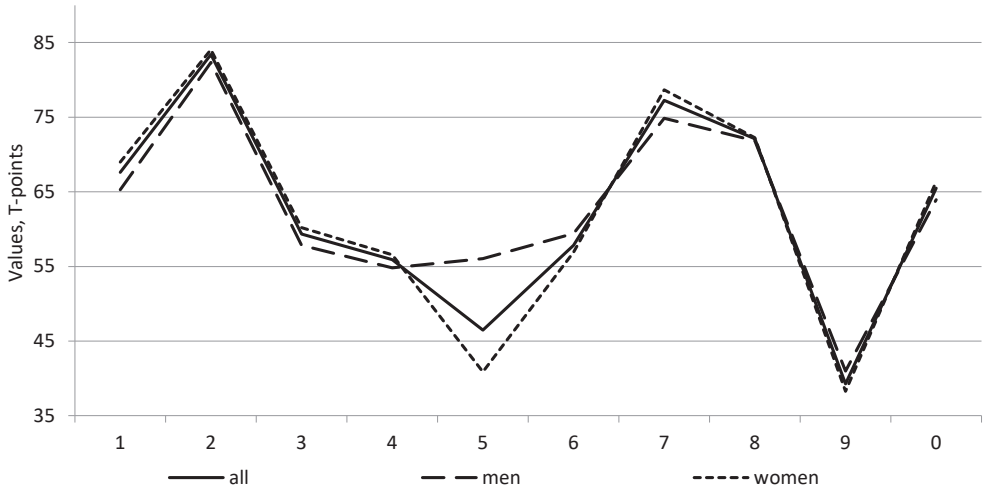
The mean age of the examined patients at the time of symptomatic onset was 21.3±6.5 years old (average 19.0 years, interquartile range 17.0-22.0 years): men 20.5±5.8 years old (18.0 years, 17.0-21.0 years), women 21.9±6.9 years old (18.5 years, 18.5-22.5 years); the age at the time of seeking medical advice and examination was 21.4± 6.4 years old (19.0 years, 18.0-22.0 years): 20.7±5.7 years old (18.0 years, 17.0-21.0 years) and 22.0±6.9 years old (19.0 years, 18.5-22.5 years), respectively.

The examined men and women were divided into three groups depending on the clinical variant of the course of PE of BAD: with the prevalence of depressive symptoms (depressive variant), 119 people (44 men and 75 women); with prevalence of manic or hypomanic symptoms (manic variant), 23 persons (15 men and 8 women); and with simultaneous presence of depressive and manic symptoms, or with rapid phase change (mixed version), number of 11 persons (6 men and 5 women).

Results

Individual-psychological profile of patients with depressive variant of PE of BAD is characterized by dominance of signs of depression in combination with symptoms of anxiety and fatigue (Fig. 1).

The average values of the indicators were: by the scale of overcontrol – 67.61±8.56 points: 65.30±9.51 points in men and 68.97±7.69 points in women ($p<0.05$); pessimism – 83.40±4.08 points; 82.32±3.87 points and 84.04±4.09 points ($p<0.05$), respectively; emotional lability – 59.33±7.88 points: 57.84±8.25 points and 60.20±7.57 points ($p<0.05$) respectively; impulsivity – 55.92±6.12 points: 54.82±5.27 points and 56.57±6.51 points ($p>0.05$) respectively; masculinity-femininity – 46.48±8,88 points: 56.05±2.97 points and 40.87±5.86 points ($p<0.01$) respectively; rigidity – 57.84±5.44 points: 59.41±5.04 points and 56.92±5.49 points ($p<0.05$) respectively; anxiety – 77.25±6.50 points: 74.86±7.85 points and 78.65±5.12 points ($p<0.05$) respectively; individuality – 72.09±1.03 points: 71.82±0.84 points and 72.25±1.10 points ($p>0.05$) respectively; optimism – 39.27±5.91 points: 40.98±9.42 points and 38.27±1.13 points



1 – Overcontrol; 2 – Pessimism; 3 – Emotional lability; 4 – Impulsivity; 5 – Masculinity-femininity; 6 – Rigidity; 7 – Anxiety; 8 – Individuality; 9 – Optimism; 0 – Introversion

Fig. 1. Standardized multifactor method of personality research profiles for Men and Women with depressive primary episode of bipolar affective disorder.

(p<0.05) respectively; introversion – 65.48±3.99 points: 63.95±4.01 points and 66.37±3.72 points (p<0.01) respectively.

Fig. 1 shows that the profiles of men and women with the depressive variant of PE of BAD are similar, although statistical and mathematical analysis of indicators proves significant differences on the main scales. The profiles of both men and women are dominated by 2 scales ('pessimistic'), with its average values exceeding 80 T-points, which indicates a psychotic level of depressive manifestations. High values on the pessimistic scale are depressive manifestations, expressiveness of affiliative need with its frustration, blocking of activity with refusal of self-realization. It should be noted that high scores on the pessimistic scale are not only an indicator of the current depressive state of a situational or endogenous character, but also as a predictive factor for a depressive response as a universal pattern of psychological response of an individual in situations of stration, distress or disadaptation. Tendencies revealed by high quantitative values on the pessimistic scale are exacerbated by high (over 70 T-points) values on the scale 7 ('anxiety') and extremely low (about 40 T-points) indicators on the scale 9 ('optimism'). Such a profile can be interpreted as a manifestation of anxious-thinking tendencies against the acutely reduced mood (in this contingent – endogenous character) with inhibition of activity, desire to stop all activities, lack of energy and increase of feeling of exhaustion and fatigue. The parallel rise on the scale 8 ('individuality') fits into the psychological pattern of hypoactivity, with a predominance of concentration on internal experiences and feelings over external activity, internal tension and anxiety, fixation on problems and the expectation of deterioration. It is also worth mentioning a rather high (over 65 T-points) indicators on the scale 1 ('overcontrol'), which reflect the fixation on unpleasant somatic sensations, which quite often accompanies depression development. In the profile of patients with depressive variant of PE of BAD the described tendencies are combined with high rates on the scale 0 ('introversion') that evidences of hypostenic manifestations, passivity of personal position, fixation on internal experiences, decrease of involvement in social environment, decrease in the number of social contacts; high scores on this scale can also be an indicator of response to current difficulties and displaying escapism.

Low rates on scale 4 ('impulsivity') of the profile of patients with depressive variant appropriately reflects the decrease of motivation achievement, developing manifestations of inhibition, reducing overall energy potential, and demonstrates intensive formation of vital apathy, depressive and asthenic-depressive pattern rather than agitated or dysphoric. In the profile of patients with depressive PE of BAD, they are combined with low scores the scale 3 ('emotional lability'), which reflects the staticness of depressive tendencies, desire to decrease activity focus on internal experiences, general inhibition. Increasing rates on the scale 5 ('masculinity-femininity') in men can be interpreted as suppression of sexual activity under the influence of depression, sentimentality, sensitivity, vulnerability. In women with depressive PE of BAD, the SMMPR scores on the scale 5 are reduced, indicating a decrease in libido, high sensitivity, desire to be protected, asthenic-depressive mood.

The SMMPR profile of patients with the manic variant of PE of BAD is significantly different from that inherent in the patients with a depressive variant (Fig. 2). The mean values of the indicators on the scales were: over control – 55.22±3.29 points, 53.93±2.49 points and 57.63±3.38 points (p<0.05) respectively; pessimism – 42.30±6.10 points, 40.40±5.46 points and 45.88±5.91 points (p<0.05) respectively; emotional lability – 55.61±4.60 points, 53.93±3.20 points and 58.75±5.37 points (p<0.05) respectively; impulsivity – 78.48±4.45 points, 79.87±4.02 points and 75.88±4.26 points (p<0.05) respectively; masculinity-femininity – 55.43±14.94 points, 45.13±3.68 points and 74.75±4.53 points (p<0.01) respectively; rigidity – 67.61±5.69 points, 69.60±5.80 points and 63.88±3.18 points (p<0.05) respectively; anxiety – 52.87±4.41 points, 51.47±4.09 points and 55.50±3.96 points (p<0.05) respectively; individuality – 53.52±9.46 points, 53.87±9.33 points and 52.88±10.32 points (p>0.05) respectively; optimism – 75.57±3.89 points, 76.73±3.49 points and 73.38±3.85 points (p<0.05) respectively; introversion – 52.83±8.49 points, 50.33±7.20 points and 57.50±9.21 points (p<0.05) respectively.

Profile analysis proves predominance of individually-psychological characteristics of the patients with manifestations of mania and impulsivity. High (70 T-points) figures on the scales of optimism and impulsiveness reflect elevated mood, bright emotions high level of activity (usually chaotic and spontaneous),

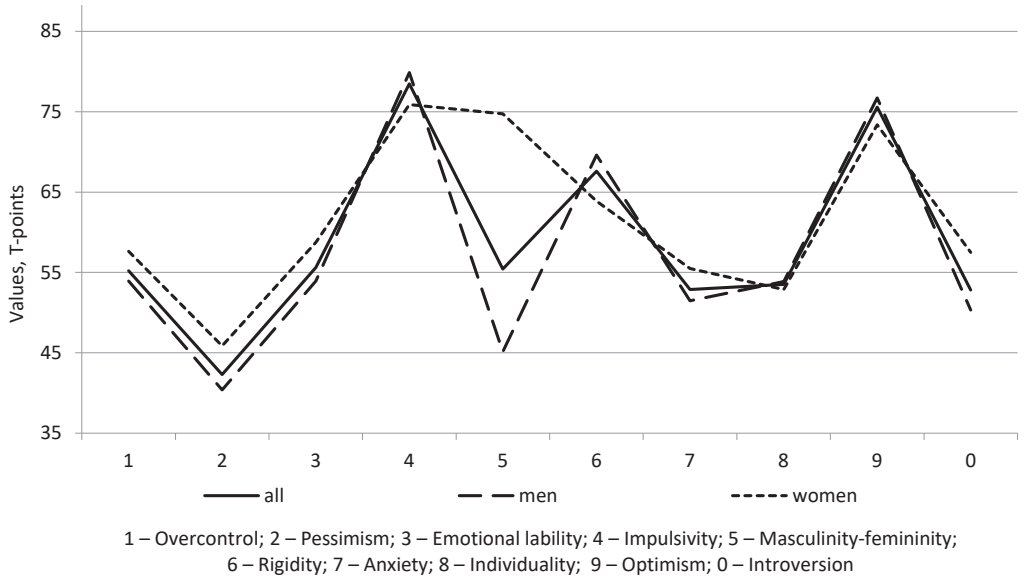


Fig. 2. Standardized multifactor method of personality research profiles for Men and Women with Manic primary episode of bipolar affective disorder.

inflated self-esteem, ease decision making promiscuous intercourse, arrogance in communication, impaired control, easy conflict, reduction of criticality to one's condition, readiness to act impulsively and hastily. Such a profile is associated with coping strategies for displacing unpleasant information, combined with intolerance and aggression towards its source, with difficulty in self-control. It should be noted that in the examined patients high scores on the scales of impulsivity and optimism are combined with high (over 60 points) indicators on the scale 6 ('rigidity') that might be an indicator of explosive type of reaction with a tendency to uncontrolled reactions of aggression, domination, rejection of opposition from the environment, beliefs in their own right and exclusivity up to a delusion of grandeur. In the profiles of patients with a manic variant of PE of BAD, the peak increase of indicators on the scales of impulsivity, optimism and rigidity is accompanied by a significant decrease in indicators on the scales of pessimism, overcontrol, anxiety, emotional lability and introversion. Relatively, in the psychological pattern of response, the signs of sensitivity to other people's thoughts are suppressed, attention to one's own state of health is not typical (even to the complete disregard of existing problems), lengthy reflections with situation analysis, reflection and introspection. Such trends create a favorable ground for deviant behavior, first of all addictive and delinquent ones. The decreased scores on the scale 5 for Men is a manifestation of sexual aggravation and increased sex drive associated with a manic state, indiscriminate sexual contact, ignoring commonly accepted moral rules and regulations.

Moreover, the individual psychological profile in the patients with mixed variant of PE of BAD is characterized by originality and differs from the profiles of patients with depressive and manic variants (Fig. 3). The average values on the scales in the patients with mixed variant of PE of BAD were: over control – 60.36±10.98 points, 54.67±10.33 points and 67.20±7.82 points ($p>0.05$) respectively; pessimism – 68.09±8.25 points, 62.50±2.81 points and 74.80±7.56 points ($p<0.05$) respectively; emotional lability – 60.91±6.61 points, 56.67±4.59 points and 66.00±4.85 points ($p<0.05$) respectively; impulsivity – 66.09±7.08 points, 70.50±3.56 points and 60.80±6.72 points ($p<0.05$) respectively; masculinity-femininity – 54.91±13.88 points, 46.17±5.88 points and 65.40±13.65 points ($p<0.05$) respectively; rigidity – 60.82±5.33 points, 64.33±4.50 points and 56.60±2.19 points ($p<0.05$) respectively; anxiety – 63.27±11.23 points, 56.67±7.20 points and 71.20±10.31 points ($p<0.05$) respectively; individuality – 65.82±6.71 points, 66.67±6.59 points and 64.80±7.46 points ($p>0.05$) respectively; optimism – 56.64±14.38 points, 65.17±9.33 points and 46.40±12.97 points ($p<0.05$) respectively; introversion – 58.27±9.55 points, 52.33±8.33 points and 65.40±4.98 points ($p<0.05$) respectively.

In the profile of men with mixed PE of BAD impulsiveness (4 scales) predominates,

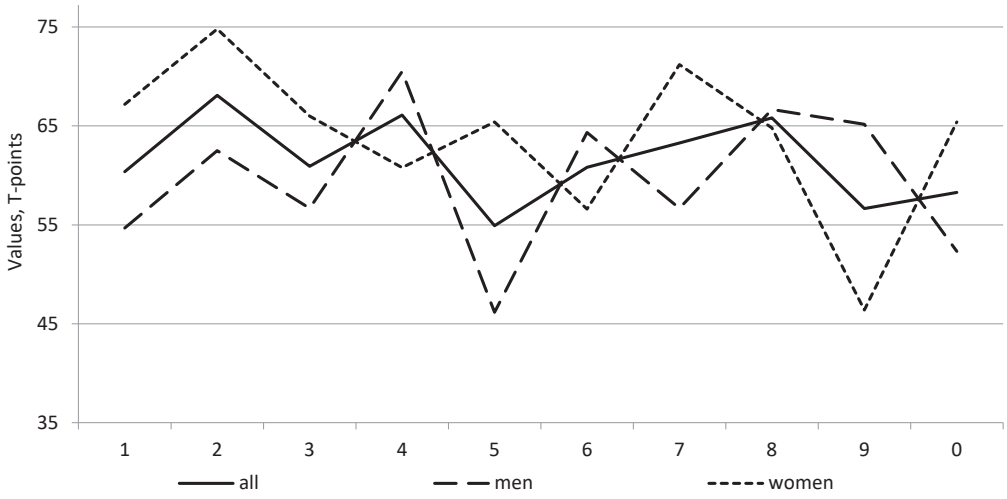


Fig. 3. Standardized multifactor method of personality research profiles for Men and Women with mixed primary episode of bipolar affective disorder.

although the quantitative values of the indicators are smaller than in the manic variant of PE of BAD. In men, increased (more than 65 T-points) indicators on the impulsivity scale are combined with increased indicators on the scales of individuality, hypomania and rigidity. Such a pattern is a manifestation of the instability of the emotional state, a tendency to mood swings, unconformity, originality and non-template reactions to external challenges, a tendency to impulsiveness, conflict, psychopathization of personality traits. At the same time, the combination of contradictory traits is a constant source of intrapsychic conflicts, which is manifested by high excitability and dynamism combined with inertness and instability of behavior. High activity in such individuals is combined with rapid fatigue and self-doubt, which, under certain conditions, can create an addictive predisposition. They are characterized by increased irritability and offensiveness, hostility combined with conflict and hostility to others, especially expressed in frustration of urgent needs. In the men's profiles with the mixed version of PE of BAD, repressive positions occupy the scales of masculinity-femininity, overcontrol, emotional lability, anxiety and introversion, which are indicators of sexual behavior disorders, inhibition, loss of control over impulsivity, weakness of the emotional state. In general, it can be argued that the individual-psychological profiles of SMMPR in men with mixed PE of BAD are more closely related to the profiles of the patients with manic variant than with depressive one, although they have specific features.

Discussion

In recent years, much attention is paid to the restoration of social functioning and the quality of life of the mentally ill. Quality of life, which reflects the main aspects of the mental, social and physical functioning of the patient, is a key criterion for evaluation of the effectiveness of the health care in psychiatry [14]. Thus, longitudinal studies revealed a significant decrease in quality of life in the patients with BAD; it is established that the patients with 25 years of disease without adequate treatment can lose 9 years of life [15]. Numerous studies have established significant social disadaptation of the patients with BAD, i.e. reduced levels of social functioning, reduced professional status and material level, difficulties in personal and professional life [16].

A thorough study is needed for the clinical and psychopathological phenomenology of the primary episode of BAD to determine the features of different variants of its course and to develop a system for predicting its clinical course.

Improving the system of diagnostic measures in the primary episode of BAD in order to improve the quality of treatment, ensure a stable remission and reduce disease recurrence, restore social functioning and quality of life for patients is of a topical scientific and practical matter [17].

Thus, the most significant differences in the quantitative indicators of SMMPR are established when comparing depressive and manic, as well as depressive and mixed variants of PE of BAD, and lesser – when comparing manic

and mixed variants. Most of all, these differences are expressed in pessimism, impulsiveness, individualism and optimism. Understanding personal features of the patients with a primary episode of BAR is an effective way of solving the problem of BAR and is of great scientific and practical importance for its predicting, treating and preventing.

Conclusions

Patients with primary episode of bipolar affective disorder have some individual psychological characteristic features that have significant gender differences, as well as differ in different variants of the debut of the disease. In the depressive variant of primary episode of bipolar affective disorder, individually psychological pattern is characterized by a tendency to asthenic variant of reaction with prevalence of affiliation, anxiety, pessimism, desire for escapism and minimization of activity. In the manic version of primary episode of BAD, the personality profile is characterized primarily by impulsivity, low level of reflectivity, aggressiveness and intolerance to the opinion of others. The mixed variant is characterized by the most complex and contradictory characteristics, reflecting the instability of emotional state with predominance of manifestations of impulsiveness and rigidity in men, and depressive and anxious traits in women. Gender differences are more significant in women with signs of pessimism, emotional lability, anxiety and introversion, and in men – of impulsiveness, rigidity and optimism.

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Conflict of Interests

The author declares no conflict of interest.

ІНДИВІДУАЛЬНО-ПСИХОЛОГІЧНІ ОСОБЛИВОСТІ ХВОРИХ З ПЕРВИННИМ ЕПІЗОДОМ БІПОЛЯРНОГО АФЕКТИВНОГО РОЗЛАДУ

Ю.І. Мисула

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Біполярний афективний розлад (БАР) – надзвичайно актуальна проблема сучасної психіатрії. Особливості первинного епізоду (ПЕ) захворювання надзвичайно важливі для прогнозування та планування лікувально-реабілітаційних заходів БАР. Індивідуально-психологічні особливості хворих на ПЕ БАР залишаються невивченими, що ускладнює розробку сучасних методів прогнозування, лікування та профілактики даного розладу.*

Мета. *Дослідити індивідуально-психологічні особливості хворих з первинним епізодом біполярного афективного розладу з урахуванням гендерного фактору і клінічного варіанту дебюту БАР.*

Методи. *Обстежено 153 пацієнта (65 чоловіків і 88 жінок) з ПЕ БАР. Обстежуваних пацієнтів розділили на три групи залежно від клінічного варіанту перебігу ПЕ БАР: депресивний, маніакальний варіант та змішаний варіант. Обстеження проведено з використанням Стандартизованого багатофакторного методу дослідження особистості (СМДО). Статистична обробка отриманих даних проводилася з використанням непараметричного тесту Манна-Уїтні.*

Результати. *Найбільші відмінності при порівнянні кількісних показників СМДО виявлені при порівнянні депресивного і маніакального, та депресивного і змішаного варіантів ПЕ БАР, і менші – при порівнянні маніакального та змішаного варіантів. Найбільшою мірою ці відмінності виражені для проявів песимістичності, імпульсивності, індивідуалістичності та оптимістичності.*

Висновки. *Були знайдені особливості пацієнтів чоловіків та жінок з депресивними, маніакальними та змішаними варіантами ПЕ БАР, які можуть допомогти знайти методи прогнозування, лікування та профілактики БАР.*

КЛЮЧОВІ СЛОВА: **біполярний афективний розлад; індивідуально-психологічні особливості; депресивний, маніакальний та змішаний варіанти.**

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DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF MICROBIAL FLORA IMBALANCE IN GINGIVAL BIOFILM

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Background. *Periodontal tissues inflammatory diseases are widespread among young people.*
Objective. *This study was aimed at elaborating the method to assess risks of periodontal inflammatory diseases and determining its efficacy depending on the state of dental tissues, gum tissues and sex.*
Methods. *The study included 182 students (93 men, 89 women) aged 19-29: 22 individuals had no lesions of hard dental tissues and no signs of periodontal disease; 51 individuals were found to have DMF index <6; 52 individuals – DMF index ≥6; 57 individuals were diagnosed with chronic catarrhal gingivitis. Primary groups were formed in autumn; re-examination was carried in spring. The research participants were assessed for detection of risks of periodontal inflammatory disease by the method developes by the authors (Patent UA 54041).*
Results. *The study revealed that the risk of development of preiodontitis increases in individuals with high caries and gingivitis intensity. In spring, more individuals suffer from microbial imbalance in in the composition of gingival sulcus fluid and decrease in the mean stability coefficient value that indicates an increased risk of inflammatory periodontal disease development. Women were less likely to experience seasonal dysbiotic changes in the gingival sulcus fluid composition compared with men.*
Conclusions. *The method suggested for assessment of the risk of periodontal inflammatory diseases is of high informativeness. It allows clinicians detecting early pre-nosological signs of oral microbiocenosis imbalance that enhances the effectiveness of early diagnosis of inflammatory periodontal diseases.*
KEY WORDS: **biofilms; microflora; gingivitis; risk of morbidity.**

Introduction

According to the recent reports, oral and dental health has significantly improved in most countries, but the prevalence of inflammatory diseases of periodontal disease is still high [7, 9, 10]. Inflammatory diseases of periodontal tissues are reported to be quite common among young population [7]. These diseases are in the focus of researchers and clinicians as they are a main cause of tooth loss as well as they increase risks of systemic pathologies even in young age. Therefore, early diagnosis of inflammatory gum diseases and prognosis of their outcomes are one of topical issues of contemporary dentistry.

The oral cavity is an ecological system harbouring various types of microorganisms forming a biofilm [1,8,11,13]. In the oral cavity, bacteria can be present in the planktonic state (e. g., in saliva) and can develop as colonies that adhere to organic structures and build up plaque, and are able to organize associations for joint survival. The colonies may develop their complex and unexpected distinct properties. According to the current interpretation, the bacterial plaque is called a biofilm, which is a specialized bacterial ecosystem that provides the viability and preservation of microbial species forming the biofilm and promotes their general population increase [14]. Moreover, biofilm is an independent and self-regulating biological system, far from being an amorphous association of different bacteria.

At present, early pre-nosological signs of the risk of inflammatory gum diseases are hardly diagnosed in dental practice. The relations between the early micro-ecological imbalance of the oral cavity and risk of gingival inflammation development are still unclear as well as the issues of gum disease prediction. The development of accessible and easy-to-use methods for early diagnosis and prediction of gum diseases and their progression in young age allows clinicians providing an evidence-based approach to choose the proper tactics in managing such patients.

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This study was aimed at elaborating the prognostic criteria to assess risks of periodontal inflammatory diseases and determining its efficacy depending on the state of dental tissues, gum tissues and sex.

Methods

182 students of a medical college aged 19-29. 22 individuals (11 men and 11 women), who had no lesions of hard dental tissues and no signs of periodontal disease, made up the control group. 51 individuals (26 men, 25 women) were found to have DMF index <6; 52 individuals (27 men, 25 women) – DMF index ≥6; 57 individuals (29 men, 28 women) were diagnosed with chronic catarrhal gingivitis. Primary groups were formed in autumn (October-November); re-examination of the test groups was carried out in 6 months in spring (April-May).

The study was conducted in accordance with the Helsinki Declaration of the World Medical Association on the ethical principles for medical research involving human subjects [15]. Signed written informed consents to participate in research study were given by research project participants that was an indispensable condition for the inclusion of students in the study.

The research participants underwent standard dental clinical examination to determine caries intensity index (DMF index), oral hygiene index (Greene-Vermilion index) (OHI-S), PMA gingival index modified by C. Parma, Muhlemann bleeding index, Muhlemann-Saxer index (PBI), interdental hygiene index (HYG), complex periodontal index (CPI). All subjects were assessed for the risk of periodontal inflammatory disease by the method elaborated by the authors – the Patent (utility model UA 54041, published Information Bulletin) [2].

The method of assessment of the risks of periodontal inflammatory diseases development is: gingival fluid is obtained in 1-2 hours following tooth brushing with a sterile paper pin of 10 mm long, by inserting its end in the orifice of the gingival groove. When the paper pin gets soaked with the fluid of the gingival groove, it is placed in 0.1 ml of sterile saline and washed thoroughly. After that this saline suspension of microorganisms is put onto degreased sterile slide with following drying, fixing, staining by Gram techniques. Immersion microscopy is used to count the number of Gram-positive cocci, Gram-negative cocci, Gram-positive rods, Gram-negative rods, Gram-negative spirilla as a percentage of total bacterial cells counted.

The stability coefficient (SC) is calculated by the ratio of the sum of the number of Gram-positive cocci and Gram-positive rod-shaped microorganisms as percentage to the sum of the number of Gram-negative rods and Gram-negative spirilla as percentage. When the SC value equals 2-4, this indicates the ecological balance between bacterial populations, prevalence of symbiotic stabilizing microbiota, and no risks of inflammatory periodontal disease. When the SC value is >4 (SC shift to the right), this points out an increase in the number of Gram-positive bacteria residing on the gum tissues. These microorganisms are constituents of the dental plaque and contribute to development of inflammatory response characteristic of gingivitis, that is, the risk of inflammatory periodontal diseases increases. When the SC value is <2 (SC shift to the left), this evidenced an increase in obligatory anaerobic Gram-negative rod-shaped bacteria (bacteroids) and spirilla that have periodontopathogenic effect, i.e., the risk of periodontitis development increases [2].

Statistical analysis of the findings obtained was carried out using the SPSS 17.0 and Microsoft Excel 2003 programs. The obtained quantitative indicators were processed by the methods of mathematical statistics with definition of mean values (M) and errors of mean values (m) in the groups of individuals under the study. The statistically significance of differences between the investigated indicators was estimated by the Student's t-test criterion. For comparison of the particles in separate groups, χ^2 criterion was used to determine the statistical significance of their differences.

Results

The assessment of the risk of inflammatory periodontal diseases that was carried out in the autumn has revealed the following. In the control group, the incidence rate of SC within the range of 2-4 made up 95.5%, in 4.5% of individuals there was a SC shift to the right (Fig. 1). The development of caries was accompanied by changes in the frequency structure of the SC gradations. In the individuals with DMF index <6, the number of people with SC= 2-4 decreased by 34.7%; the SC shift to the left was observed to be as more often as by 29.4%, and the frequency of the SC shift to the right increased by 5.3% (χ^2=97042.761, p=0.0001). The increase in the caries intensity to DMF index ≥6

was accompanied by a decrease in the number of individuals with SC=2-4 to 53.8%; 25.0% of the subjects were found to have SC<2, the number of individuals with SC shift to the right increased to 21.2% (χ^2=71518240, p=0.0001). Among the patients with gingivitis, subjects with SC<2 (73.7%) and SC>4 (26.3%) prevailed, while the patients with SC=2-4 were not detected (χ^2=648866.373, p=0.0001).

The mean SC value decreased in the subjects with DMF index <6 by 18.2% (2.92±0.18 vs. 3.57±0.11 in control, p=0.002) and especially in patients with gingivitis, by 38.7 % (2.19±0.20, p=0.0005).

The regularities of the frequency in detecting certain SC gradations and their absolute values did not depend on sex.

In spring, the frequency of detecting SC gradations depended on the condition of teeth and gums (Fig. 2). The number of individuals with dysbiotic shifts in the gingival fluid microbiota in comparison with the control group, increased in the individuals with DMF index <6 by 6.2% (χ^2=3876.859, p=0.0001), in the individuals with DMF index ≥6 by 16.8 % (χ^2= 8653.959, p=0.0001), and in the patients with gingivitis there was an increase by 59,1% (χ^2=13941.459, p=0.0001). The mean SC value in the individuals with caries did not differ from those in the control group (2.54±0.16 for DMF index <6; 2.82±0.19 for DMF index ≥6 vs. 2.62±0.19 for the control). The mean SC value in the individuals with catarrhal gingivitis was 1.6 times lower compared with the control (1.67±0.17, p=0.0004).

In spring, compared to the fall, the frequency profile in all the study groups changed due to an increase in the number of individuals with SC shift to the left: in the control group, it increased by 40.9% (χ^2=77307.546, p=0.0001), in the individuals with DMF ≥6 – by 15.4% (χ^2=17.484, p=0.0001), and in the individuals with catarrhal gingivitis – by 15.8% (χ^2=7.329, p=0.007). The mean SC value decreased in the control group

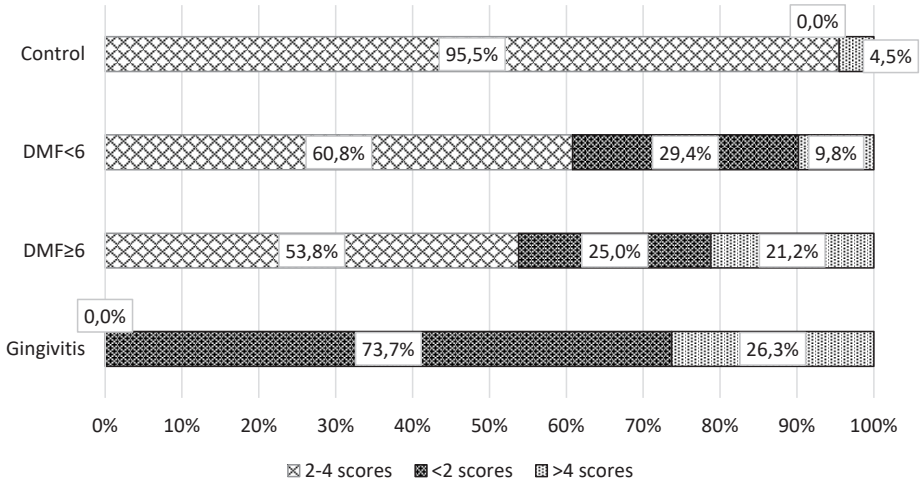

Fig. 1. Rate of SC gradations in adolescents in autumn.

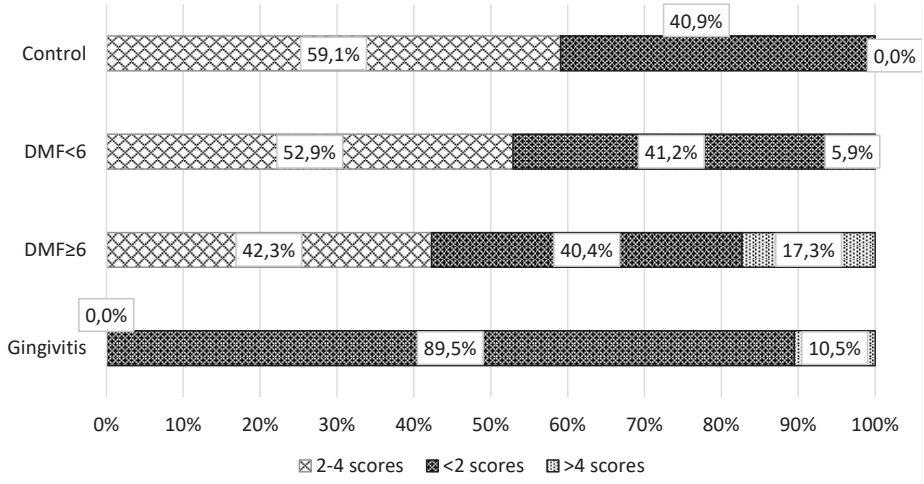

Fig. 2. Rate of SC gradations in adolescents in spring.

in 1.4 times (p=0.0001), and in the patients with gingivitis – in 1.3 times (p=0.047).

In spring, in men and women, the SC frequency profile also depended on the dental status and was characterized by a significantly higher number of individuals with dysbiosis in the gingival sulcus biofilm in all the studied groups compared to the control. The mean SC value in men with gingivitis was 1.6 times lower than in the control group (1.61±0.24 versus 2.59±0.26 in control, p=0.019), while in women it did not differ significantly (1.77±0.24 vs. 2.64±0.27 in the control).

In spring, in comparison with autumn, the number of persons with SC shift to the left increased in both men and women: in the control groups of men it increased by 45.5% (χ^2=22721.819, p=0.0001), in women – by 34.6% (χ^2=15994.909, p=0.0001); in men with DMF index ≥6, it increased by 22.3% (χ^2=6.9661, p=0.031), in women with gingivitis – by 17.9% (χ^2=4.375, p=0.036). The mean SC value in the control groups decreased in men in 1.4 times (2.59±0.26 in the spring compared with 3.71±0.12 in autumn, p=0.002), and in women, it – in 1.3 times (2.64±0.27 in spring compared to 3.43±0.18 in autumn, p=0.028).

In spring, male subjects showed gradations of SC<2 and SC>4 more often than female subjects, and in particular, in the male patients with high caries intensity, it was observed to be more frequent (by 26.4%) (χ^2=8.397, p=0.015). The attained results testify that women are less likely to experience seasonal dysbiotic changes in the gingival sulcus biofilm.

In the individuals, whose gums were assessed as intact in autumn, the inflammation development in spring was detected: in the control group, there were 3 individuals with signs of inflammation (1 woman, 2 men) constituting 13.6% of the whole group, in the adolescents with DMF index <6, there were 16 individuals (9 men, 7 women) that made up 31.4% of the relevant group; and in the individuals with DFF index ≥6, there were 21 patients (14 men, 7 women) that made up 40.4% of the individuals. The highest periodontal indexes were observed in the patients with catarrhal gingivitis, but in the patients with caries, their PMA, Muhlemann, and PBI indices were significantly higher than those of the control group. In the study group of patients with catarrhal gingivitis in spring, there was a tendency to increase in the periodontal indices, compared with the autumn. In particular, the PMA was 27.7±0.89% (compared with 0.91±0.51% in the control group), the Muhlemann index – 1.45±0.057 (in the control 0.02±0.013), the PBI index – 0.91±0.034 (0.01±0.006 in the control group). In the patients with caries, PMA, Muhlemann and PBI indexes were also significantly higher than the controls. Thus, in persons with DMF <6 PMA, this index was in 3.9 times higher in the control group (p<0.05), the Muhlemann index – in 3.0 times (p<0.05), and the PBI index – in 6.0 times (p<0.05). In the patients with DMF ≥6, the periodontal indexes were even higher than the corresponding control group values, namely: PMA – in 4.6 times (p<0.05), Muhlemann index – in 4.0 times (p<0.05), the PBI index – in 7.0 times (p<0.05).

In the study group of patients with catarrhal gingivitis in spring a tendency to increase in the periodontal indices was observed, compared with the autumn season.

In men and women, in spring, the changes in indexes compare to the groups by dental status and by season had the same features as in the groups without regard to sex.

Discussion

The study revealed an increase in the incidence of inflammatory processes in periodontal tissues of adolescents in spring. A number of reports have emphasized polyetiological nature of periodontal diseases. And in addition to that, inflammatory reactions provoked by gingival biofilm microflora are established to greatly contribute to development of periodontal diseases [5,6,12]. The suggested criteria have demonstrated an increase in the incidence of dysbiosis in the gingival biofilm in adolescents in spring that, in our opinion, determine an increase in the incidence of gingivitis in spring compared with autumn.

The quantitative and specific composition of the oral microbial flora of each healthy individual is relatively stable, since there are a number of factors that maintain its constancy. The most important factor in maintaining the stability of the oral microbial composition is the antagonism inherent in the resident microbial flora relative to pathogenic and opportunistic microorganisms, when a stable microbial community crowds out pathogenic agents from the oral cavity [13].

Compensatory properties of symbiotic microbial flora are far from being limitless and under the influence of various factors, the dynamic equilibrium between a normal and pathogenic flora may be disrupted [3,4]. For instance, the disorders of swallowing, chewing

and salivation always lead to an increase in the number of pathogenic microorganisms in the oral cavity. As a result, sharp suppression of normal microbial flora representatives occurs, i.e. dysbiosis develops, that means qualitative and/or quantitative changes in the resident microbial flora resulting from the impact of various exogenous or endogenous factors on the body [5].

Conclusions

The suggested prognostic criteria for assessing the risk of periodontal inflammatory diseases are of high informativeness, they allow clinicians detecting early pre-nosological signs of oral microbiocenosis imbalance that enhances the effectiveness of early diagnosis of inflammatory periodontal diseases, and can be used as a marker to evaluate the degree of body adaptation to the environment factors. The imbalance of indigenous and periodontopathogenic microbial flora has a significant impact on the oral status of adolescents regardless of sex. The risk of periodontitis development increases in individuals with caries severity and gingivitis intensity. In spring, more individuals were identified to have microbial imbalance in the composition of gingival sulcus fluid and a decrease in the mean stability coefficient that indicates an increase in the risk of inflammatory periodontal disease development. Women were less likely to experience seasonal dysbiotic changes in the gingival sulcus fluid composition compared with men.

In clinical dental practice early diagnosis of periodontal diseases and prediction of their development is significant for elaborating effective preventive measures. To predict the probable progression of the disease, taking into account the patterns of pathological processes and the course of the disease, the attained results require wider applying of mathematical analysis (correlation and regressive). Detection of a wide range of relations not only improves the effectiveness of personalized prediction of microecological imbalance in the oral cavity but also allows choosing and prescribing appropriate preventive therapy or start treatment at the right time.

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Conflict of Interests

The authors declare no conflict of interest.

Authors Contributions

Loban' G.A. – conceptualization, methodology, project administration, writing – review & editing; *Petrushanko T.O.* – methodology, project administration; *Chereda V.V.* – investigation, visualization; *Faustova M.O*. – formal analysis, writing – review & editing; *Ananieva M.M.* – visualization, writing – original draft; *Basarab Ya.O.* – writing – original draft.

ДІАГНОСТИЧНЕ І ПРОГНОСТИЧНЕ ЗНАЧЕННЯ ДИСБАЛАНСУ МІКРОФЛОРИ ЯСЕННОЇ БІОПЛІВКИ

Г.А. Лобань, Т.О. Петрушанко, В.В. Череда, М.О. Фаустова, М.М. Ананьєва, Я.О. Басараб

УКРАЇНСЬКА МЕДИЧНА СТОМАТОЛОГІЧНА АКАДЕМІЯ, ПОЛТАВА, УКРАЇНА

Вступ. *Запальні захворювання тканин пародонта широко поширені серед молоді.*

Мета. *Це дослідження було спрямоване на розробку методу оцінки ризику запальних захворювань пародонта та визначення його ефективності залежно від стану тканин зубів та ясен і гендерного фактору.*

Методи. *Обстежено 182 студенти (93 чоловіків і 89 жінок) віком 19-29 років, з яких 22 особи не мали уражень твердих тканин зубів та пародонта, 51 особа з рівнем КПВ<6, 52 особи з рівнем КПВ≥6, 57 осіб з діагностованим хронічним катаральним гінгівітом. Первинні групи були сформовані восени, повторне обстеження проводилося навесні. В усіх досліджуваних провели визначення стоматологічного статусу, виявлення ризику розвитку запальних захворювань пародонта здійснили за власною методикою (патент UA 54041).*

Результати. *Проведені дослідження показали, що ризик розвитку пародонтиту підвищується в осіб з високою інтенсивністю карієсу та гінгівітом. У весняний період року виявили більшу кількість осіб з дисбалансом біоплівки ясенної борозни та зниження середньої величини коефіцієнту сталості порівняно з осіннім сезоном, що свідчило про збільшення ризику розвитку запальних захворювань*

пародонта навесні. У жінок рідше спостерігали сезонні дисбіотичні зміни біоплівки ясенної борозни порівняно з чоловіками.

Висновки. *Запропонований метод оцінки ризику запальних захворювань пародонта має високу інформативність, дозволяє виявити ранні донозологічні порушення мікробіоценозу порожнини рота, що підвищує ефективність ранньої діагностики. запальних захворювань пародонта, і може бути використаний як маркер ступеня адаптації організму до факторів зовнішнього середовища.*

КЛЮЧОВІ СЛОВА: **біоплівки; мікрофлора; гінгівіт; ризик захворювань.**

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YOUDEN'S TEST FOR CHROMATOGRAPHIC DETERMINATION OF ENALAPRIL IN PHARMACEUTICALS

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Background. *Robustness tests were firstly introduced for avoiding problems in interlaboratory studies and identifying the factors potentially responsible. A robustness test performing in late validation procedure involves the possibility that when the method is established not robust, it should be redeveloped and optimized. At this stage much effort has been made and money spent for optimization and validation, and therefore avoiding this would be great.*

Objective. *The aim of the study was to consider the robustness of HPLC determination of enalapril (in tablets) by the Youden's test.*

Methods. *Youden's test was chosen as an efficient method to assess the robustness among all analytical methods that is by means of an experiment design, which involved seven analytical parameters combined in eight tests. In previous studies, we evaluated the chromatographic method robustness to quantify enalapril (in tablets) by Youden's test.*

Results. *According to the Youden's test criteria, HPLC method proved to be greatly robust regarding the enalapril content in introduction of variation of seven analytic parameters. The lowest variation in enalapril content was 0.91 %, when Grace Platinump C8 EPS column (4.6 mm i.d. X 250 mm, 5 µm) was used. A holistic approach concerning simultaneous innovations in particle technology and instrument design was endeavored for the first time to meet and tackle the analytical laboratory issues. This was aimed at promoting success of analytical scientists as well as profitability and productiveness of business.*

Conclusion. *The Youden's test has been proved to be an efficient and useful tool for evaluation of robustness of enalapril HPLC assay.*

KEY WORD: **enalapril; high-performance liquid chromatography; robustness; quantitative analysis; Youden's test.**

Introduction

Recently, Robustness testing is best known and most commonly used in the pharmaceutics because of the stringent regulations in the domain set by regulatory authorities that requires extensively validated methods. Therefore most definitions and existing methodologies, e.g. those from the ICH, are found in the field, as stated before. Though, this has no implications for robustness testing of analytical methods in other domains and therefore this guideline is not confined to pharmaceutical methods [1].

Evaluation of robustness of chromatographic method is a laborious, complex and straining process, taking into account a great number of analytical parameters considered while carrying out the test. Some authors consider specific analytical parameters presenting small variations in the nominal conditions; statistical analysis is made using the Student's *t*-test or ANOVA test. Other alternative for evaluation of robustness of analytical methods is the Youden's test. This test assesses not only the robustness of the method but also determines the each analytical parameter effect on final results. The main idea of the Youden's test is not studying one alteration at time but introducing several changes all together in this way that the effects of individual changes can be determined [2, 3, 4].

Enalapril maleate is a maleate salt of enalapril, the ethyl ester of a long-acting angiotensin converting enzyme inhibitor, enalaprilat. Enalapril maleate is chemically defined as (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1). Enalapril, after hydrolysis to enalaprilat, inhibits angiotensin-converting enzyme (ACE) in humans and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the

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vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Enalapril in hypertension and heart failure beneficially effects primarily from suppression of the renin-angiotensin-aldosterone system. Inhibition of ACE leads to decrease of plasma angiotensin II that results in decrease of vasopressor activity and decrease of aldosterone secretion [5].

The aim of the research was to determine the robustness of HPLC (High-Performance Liquid Chromatography) method for evaluation of enalapril by means of Youden's test, and define the analytical parameters that have greater influence on the final analysis.

Methods

Enalapril maleate was presented by Farmak pharmaceuticals (Kiev, Ukraine). HPLC grade acetonitrile, sodium dihydrophosphate dihydrate, phosphoric acid were got from Merck pharamaceticals.

Instrumentation and chromatographic conditions

Agilent 1260, Grace Platinump C8 EPS column (4.6 mm i.d. X 250 mm, 5 µm). Chromatographic separation was carried out at ambient temperature (22-25 °C). The compound was separated isocratically with a mobile phase consisting of acetonitrile and buffer solution pH 2.2 (25/75, *v/v*), at a flow rate 2.0 mL/min with injection volume 50 µL. Column temperature was 50 °C. The effluent was monitored spectrophotometrically at a wavelength 215 nm.

Preparation of mobile phase

To prepare buffer solution pH 2.2.: 3.59 g of sodium dihydrophosphate dihydrate was dissolved in 1800 ml of water, the pH of the solution was fixed with phosphoric acid to the value (2.2±0.05), and then the volume of the solution with water R to 2000.0 ml was added and mixed.

Stock standard solutions

20 mg of the standard sample of enalapril maleate was dissolved in a solvent, added 0.5 ml of a solution of enalaprilat with a concentration of 0.4 mg/ml and 2.0 ml of enalapril diketopiperazine solution at a concentration of 0.4 mg/ml was adjusted to a volume of 100.0 ml with the same solvent.

Procedures

The standard solutions were prepared by dilution of the stock standard solution of mobile phase. Triplicate 50.0 µL injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph and regression equation was computed [6].

Results

The robustness assessment of HPLC method for enalapril quantitation was performed by the method suggested by Youdene Steiner. For the nominal values of the method, seven analytical parameters were chosen and minor variations were induced. After, eight runs were completed in order to determine the effect of each parameter on the final result. The seven analytical parameters as well as the variations are presented in Table 1. The analytical circumstances of the nominal values are defined by capital letters and of the small variation – by lowercase letters.

The seven parameters and their respective variations were joined into eight assays or chromatographic runs randomly performed. The factorial combination of parameters for the Youden's test is presented in Table 2. The results of the analyses are defined by the letters

Table 1. Analytical parameters and variations for the robustness evaluation of HPLC method for enalapril quantitation

Parameter		Nominal condition			Variation		
A/a	Acetonitrile in mobile phase	25	–	A	35	–	a
B/b	Buffer solution pH 2.2 in mobile phase	75	–	B	65	–	b
C/c	pH of buffer solution in mobile phase	2.2	–	C	2.7	–	c
D/d	Column temperature, °C	50	–	D	40	–	d
E/e	Mobile phase flow rate, ml/min	2.0	–	E	1.0	–	e
F/f	Column supplier	Grace Platinump C8 EPS	–	F	Nucleosil C18	–	f
G/g	Chromatograph model	Agilent 1290	–	G	HP 1100	–	g

Table 2. Factorial combination of the analytical parameters for robustness evaluation

Analytical parameter	Factorial combination							
Acetonitrile in mobile phase	A	A	A	A	a	a	a	a
Buffer solution pH 2.2 in mobile phase	B	B	b	b	B	B	b	b
pH of buffer solution in mobile phase	C	c	C	c	C	c	C	c
Column temperature	D	D	d	d	d	d	D	D
Mobile phase flow rate	E	e	E	e	e	E	e	E
Column supplier	F	f	f	F	F	f	f	F
Chromatograph model	G	g	g	G	g	G	G	g
Result	s	t	u	v	w	x	y	z

from *s* to *z*. Hence, when combination 1 was assessed, the result was *s*, for combination 2 the result was *t*, and so on.

Three injections of each sample and stan_ dard solutions at the normal concentration were administered for each combination. A 30-minute pause for system stabilization took place after alteration of chromatographic column or mobile phase composition. In each combination the assessed results were for a peak area, retention time (Rt), tailing factor (T), theoretical plates number (N) and captopril content.

The following equation was used for evaluation of the effect of the column temperature on the final analyses results:

Effect C/c=(s+u+w+y)/4–(t+v+x+z)/4Eq

The Youden's test allows definite establishing of the parameters, which have a greater influence on the results of the analyses, and control more rigorously the eventual variations of these parameters that may arise during a routine analysis.

Discussion

In this research, the first trials were aimed to find optimal chromatographic conditions. The objective of the chromatographic method development was achievement of a peak tailing factor <1.5, retention time of between 4 and 5 minutes in consort with well resolution [7-17]. In both equipment (Agilent 1290 and HP1100), the analyses of the robustness evaluation of chromatographic method were carried out simultaneously. The results were attained in eight runs to enalapril sample and standard solutions.

The effects of the parameter variations on the analysis results are presented in Table 3.

By means of the Youden's test criteria, HPLC method proved to be significantly robust as regards the content of enalapril in case of introduced variations of seven analytical parameters [18]. The lowest variation in enalapril content was 0.91 %, when column Grace Platinump C8 EPS column (4.6 mm i.d.×250 mm, 5 μm) was used.

A holistic approach concerning simultaneous innovations in particle technology and instrument design was endeavored for the first time to meet and tackle the analytical laboratory issues. This was aimed at promoting success of analytical scientists as well as profitability and productiveness of business. The Platinum™ column advantage controlled silica exposure is the dissimilarity that makes Platinum™ columns unique. Instead of thorough covering of the silica with bonded phase to hide the silica, the exposure of the silica in Platinum™ columns is controlled to provide a dual mode separation

Table 3. Effects of the analytical parameters on content and retention time (Rt) for enalapril HPLC quantitation

Effect	Content (%)	Rt (min)
Acetonitrile in mobile phase	0.15	-0.26
Buffer solution pH 2.2 in mobile phase	0.16	-0.27
pH of buffer solution in mobile phase	0.12	0.05
Column temperature	-0.05	0.05
Mobile phase flow rate	-0.03	0.05
Column supplier	0.91	-2.05
Chromatograph model	-0.04	0.11

with both polar and non-polar sites exposed to the samples. This extends polar selectivity well beyond the other reversed-phase columns and gives separations that other columns cannot.

Conclusion

Youden's test proved to be an efficient and useful tool for the robustness evaluation of HPLC method for assay of enalapril in pharmaceuticals. Therefore, Youden's test can be successfully used for the robustness evaluation for validation process of analytical methods.

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Conflict of Interests

The author declares no conflict of interest.

ЮДЕН ТЕСТ ХРОМАТОГРАФІЧНОГО ВИЗНАЧЕННЯ ЕНАЛАПРИЛУ В ЛІКАРСЬКИХ ЗАСОБАХ

Л.С. Логойда

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Випробування на робасність спочатку були введені, щоб уникнути проблем у міжлабораторних дослідженнях та виявити потенційно відповідальні фактори. Виконання перевірки надійності в кінці процедури валідації передбачає ризик того, що, коли виявиться, що метод не є надійним, його слід переробити і оптимізувати. На цьому етапі вже було витрачено багато зусиль і грошей на оптимізацію і перевірку, і тому хочеться цього уникнути.*

Мета дослідження – *визначити робасність хроматографічного визначення еналаприлу в таблетках з використанням тесту Юдена.*

Методи дослідження. *Ефективний метод оцінки надійності аналітичних методів за допомогою тесту Юдена шляхом розробки експерименту, який включає сім аналітичних параметрів, об'єднаних у восьми тестах. У дослідженнях ми оцінювали надійність хроматографічного методу для кількісного визначення еналаприлу в таблетках з використанням тесту Юдена.*

Результати. *Використовуючи критерії випробування Юдена, метод ВЕРХ показав високу надійність щодо вмісту еналаприлу при введенні варіації семи аналітичних параметрів. Найнижча зміна вмісту еналаприлу становила 0,91%, коли використовувалася колонка Grace Platinumr C8 EPS-колони (4,6 мм i.d. X 250 мм, 5 мкм). Вперше розроблено цілісний підхід, що передбачає одночасне впровадження інновацій у технології частинок та проектування приладів. Це було зроблено для того, щоб зробити вчених-аналітиків більш успішними, а підприємства - більш прибутковими та продуктивними.*

Висновки. *Тест Юдена виявився ефективним і корисним інструментом для оцінки робастності для аналізу еналаприлу методом ВЕРХ.*

КЛЮЧОВІ СЛОВА: **еналаприл; високоефективна рідинна хроматографія; робасність; кількісний аналіз; Юден тест.**

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METHODS OF METOPROLOL ANALYSIS IN DRUGS AND BIOLOGICAL FLUIDS: REVIEW AND SUGGESTIONS

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Background. *Analytical method is increasingly implemented into fundamental pharmaceutical chemistry and analysis, considering their high sensitivity, accuracy, specificity and expressiveness.*

Objective. *Metoprolol's analytical method development was the research goal.*

Methods. *The sources were world recognized journals (1990-2019) and key words used as filter were "metoprolol", "spectrophotometry" "high-performance liquid chromatography, HPLC", "quantitative analysis", "validation".*

Results. *Chromatographic methods of analysis have the highest specificity and objectivity and allow qualitative and quantitative determination of Active Pharmaceutic Ingredient (API) in combined dosage forms and biological fluids without prior components separation. The main disadvantage of the described API analysis methods is long terms from the beginning of chromatography to API release and specific solvents used as the mobile phase in HPLC. New methods development and selection such chromatographic conditions that provide high speed and high efficiency at lower pressure of the system are essential. Also, the reduction of analysis time is achieved by simplifying the conditions for sample preparation.*

Conclusions. *Analysts are constantly working on developing new analysis methods and their optimization in order to save time and consumables, which also ensures the efficiency of the developed method. There is no monograph on the substance or dosage forms of metoprolol in SPhU. Therefore, some of the developed methods should be suggested for the SPhU monograph, which is important for ensuring pharmacopoeial quality control of medicines in Ukraine.*

KEY WORDS: **metoprolol; spectrophotometry; high-performance liquid chromatography; quantitative analysis; method development; validation.**

Introduction

The quality of treatment depends much on the bioavailability of the medicinal product, which in turn is influenced by the correct and effective pharmaceutical development. Antihypertensive drugs are currently the most widely used medicines, which is associated with increase in the population with high blood pressure and those at risk for stroke and other serious diseases of the cardiovascular system. Considerable demand for antihypertensive drugs in tablet form is a prerequisite for inclusion of a large number of generic drugs in the range of industrial enterprises. The main task of pharmaceutical development for such drugs is to develop a composition that will provide bioavailability comparable to the original. The efficacy and correctness of pharmaceutical development must be confirmed by bioequivalence studies by *in vivo* and *in vitro* methods. For these purposes, effective and reliable methods for detection of API in biological fluids should be developed. It is generally accepted to use chromatographic methods for this purpose that can provide sufficient selectivity and accuracy of determination even at relatively low doses. It should be noted that today the requirements for bioanalytical techniques are framework and too broad to be universal. Therefore, making proposals for the study of individual validation characteristics for pharmacokinetic purposes is very important. There are currently no systematic studies of metoprolol.

Metoprolol succinate is a beta1-selective (cardioselective) adrenoceptor blocking agent, for oral administration, available as extended-release tablets. Metoprolol succinate extended-release tablet has been formulated to provide a controlled and predictable release of metoprolol for once-daily administration. The tablets

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comprise a multiple unit system containing metoprolol succinate in a multitude of controlled release pellets. Each pellet acts as a separate drug delivery unit and is designed to deliver metoprolol continuously over the dosage interval. The tablets contain 23.75, 47.5, 95 and 190 mg of metoprolol succinate equivalent to 25, 50, 100 and 200 mg of metoprolol tartrate, USP, respectively. Its chemical name is (±)1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt) (Fig.1). Metoprolol is a propanolamine that is 1-(propan-2-ylamino)propan-2-ol substituted by a 4-(2-methoxyethyl)phenoxy group at position 1. It acts as a beta-adrenergic antagonist, an antihypertensive agent, a xenobiotic and an environmental contaminant. It is a propanolamine, an aromatic ether, a secondary alcohol and a secondary amino compound. Metoprolol is used for a number of conditions, including hypertension, angina pectoris, acute myocardial infarction, different types of tachyarrythmia, congestive heart failure, and prevention of migraines. Due to its selectivity, metoprolol is also prescribed for off-label use for anxiety disorders. Metoprolol blocks β1 drenergic receptors in cardiomyocytes, thereby decreasing the slope of phase 4 in the nodal action potential (reducing Na^+ uptake) and prolonging repolarization of phase 3 (slowing down K^+release). It suppresses the norepinephrine-induced increase in the sarcoplasmic reticulum Ca^{2+} leak and the spontaneous SR Ca^{2+} release, which are the major triggers for atrial fibrillation.

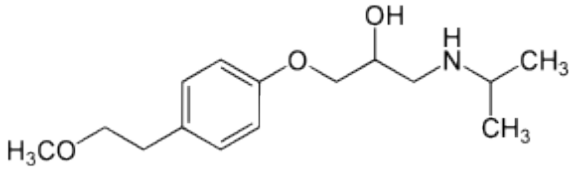

Fig. 1. Chemical structure of metoprolol.

Within the UK, metoprolol is classified as a prescription-only drug in the beta blocker class and is regulated by the Medicines and Healthcare Products Regulatory Agency (MHRA). The MHRA is a government body set up in 2003 and is responsible for regulating medicines, medical devices, and equipment used in healthcare. In the U.S. use of beta blockers such as metoprolol was approved by the Food and Drug Administration (FDA) in 1967 for the treatment of cardiac arrhythmias, hypertension, migraines, and others. Prescribers may choose to prescribe beta blockers for other treatments if there is just cause even though it is not approved by the FDA. Drug manufacturers, however, are unable to advertise beta blockers for other purposes that have not been approved by the FDA. Since the FDA does not regulate the practice of medicine after the drug has been approved, it is legal to prescribe beta blockers for other treatments such as performance anxiety. In man, absorption of metoprolol is rapid and complete. Plasma levels following oral administration of conventional metoprolol tablets, however, approximate 50% of levels following intravenous administration, indicating about 50% first-pass metabolism. Metoprolol crosses the blood-brain barrier and has been reported in the CSF in a concentration 78% of the simultaneous plasma concentration. Plasma levels achieved are highly variable after oral administration. Only a small fraction of the drug (about 12%) is bound to human serum albumin. Metoprolol is a racemic mixture of R- and S-enantiomers, and is primarily metabolized by CYP2D6. When administered orally, it exhibits stereoselective metabolism that is dependent on oxidation phenotype. Elimination is mainly by biotransformation in the liver, and the plasma half-life ranges from approximately 3 to 7 hours. Less than 5% of an oral dose of metoprolol is recovered unchanged in the urine; the rest is excreted by the kidneys as metabolites that appear to have no beta-blocking activity. Following intravenous administration of metoprolol, the urinary recovery of unchanged drug is approximately 10%. The systemic availability and half-life of metoprolol in patients with renal failure do not differ to a clinically significant degree from those in normal subjects. Consequently, no reduction in dosage is usually needed in patients with chronic renal failure. Metoprolol is metabolized predominantly by CYP2D6, an enzyme that is absent in about 8% of Caucasians (poor metabolizers) and about 2% of most other populations. CYP2D6 can be inhibited by a number of drugs. Concomitant use of inhibiting drugs in poor metabolizers will increase blood levels of metoprolol several-fold, decreasing metoprolol's cardioselectivity. Metoprolol has a very low melting point; around 120 °C (248 °F) for the tartrate, and around 136 °C (277 °F) for the succinate. Because of this, metoprolol is always manufactured in a salt-based solution, as drugs with low melting points are difficult to work with in a manufacturing environment. The free base exists as a waxy white solid, and the tartrate salt is finer crystalline material.

Metoprolol contains a stereocenter and consists of two enantiomers. Metoprolol succinate is a white crystalline powder with a molecular weight of 652.8. It is freely soluble in water; soluble in methanol; sparingly soluble in ethanol; slightly soluble in dichloromethane and 2-propanol; practically insoluble in ethylacetate, acetone, diethylether and heptane. Inactive ingredients: silicon dioxide, cellulose compounds, sodium stearyl fumarate, polyethylene glycol, titanium dioxide, paraffin. Physico-chemical analysis methods are increasingly introduced into fundamental pharmaceutical research and pharmaceutical analysis practice, taking into account their high sensitivity, accuracy, specificity and expressiveness.

Objective was analytical method development for metoprolol.

Methods

Literature survey has been done in range of years 1990-2019 to make the review updated and comprehensive and to show the new approaches to development of the methods of metoprolol analysis. The sources were world recognized journals and key words used as filter were metoprolol, spectrophotometry, high-performance liquid chromatography, quantitative analysis, method development, validation [1-5].

Results

Pharmacopeian methods of analysis of metoprolol are presented in Fig. 2. The State Pharmacopoeia of Ukraine (SPhU) has not developed a monograph on the substance of metoprolol or on the prepared medical form yet. However, the United States Pharmacopoeia regulates the determination of metoprolol succinate in extended-release tablets. For identification, IR-spectroscopy and HPLC are suggested; for quantitative determination of metoprolol succinate in tablets in assay and dissolution test – HPLC, respectively. Chromatographic conditions for determination of metoprolol succinate in tablets are specified in the monograph of the United States Pharmacopoeia, which are used the chromatographic column 4-mm×12.5-cm; 5-μm packing L7 and mobile phase consisting of acetonitrile and buffer (25:75). Mobile phase rate – 1 ml/min, detection wavelength – 280 nm, tailing factor – NMT 2.0, relative standard deviation – NMT 2.0%.

The suggested method of the United States Pharmacopeia requires a long sampling. The European Pharmacopeia has a monograph on metoprolol tartrate tablets. Identification of metoprolol tartrate of the European Pharmacopeia regulates the absorption spectrophotometry in the infrared region, UV-spectropho-

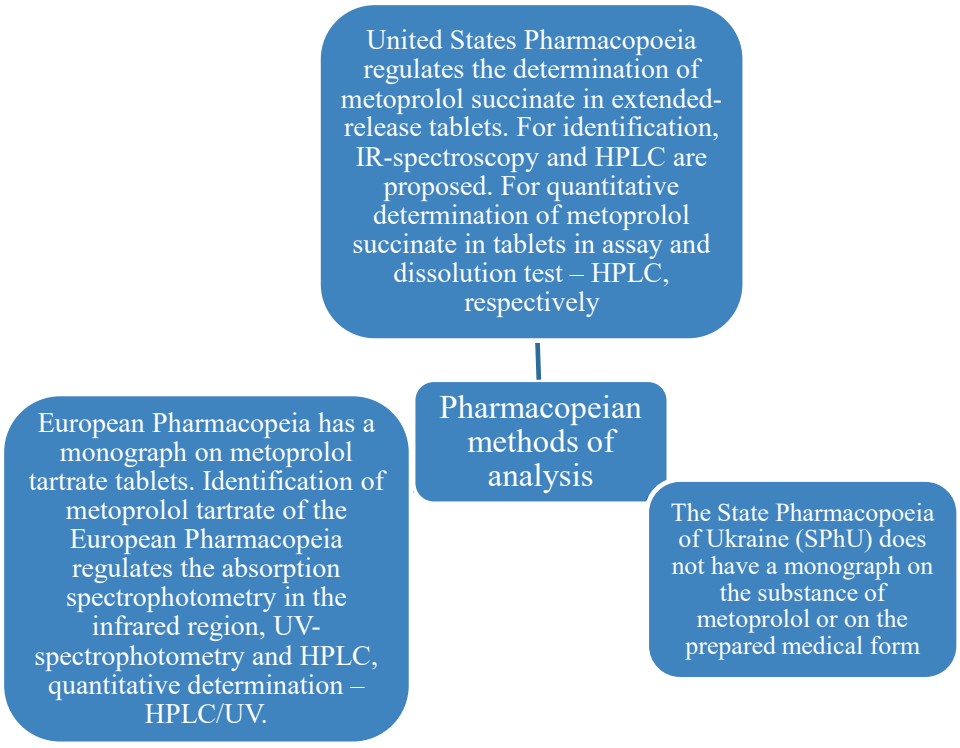

Fig. 2. Pharmacopeian methods of analysis of metoprolol.

tometry and HPLC, quantitative determination – HPLC/UV. As a solvent, a mixture of methanol and 0.1 M hydrochloric acid is used, mobile phase – solution of 1-pentanesulfonic acid sodium salt (monohydrate), anhydrous sodium acetate in mixture of methanol and water and adding glacial acetic acid. Mobile phase rate – 1.0 ml/min, detection wavelength – 254 nm.

Historical development of methods for the quantitative determination of metoprolol in substances and drugs is related to the development of analytical methods only and pharmaceutical analysis in general. Nowadays the literature contains a large number of scientific papers devoted to the quantitative determination of metoprolol and other APIs in one medical form, since metoprolol is used in combination with different APIs for the treatment of hypertension. According to the literature data, spectrophotometry [6-11], HPLC [12-21], gas chromatography [22] are the most widely used techniques for the determination of metoprolol tartrate.

Mustafa Cesme et al. suggested spectrophotometric determination of metoprolol tartrate in pharmaceutical dosage forms on complex formation with Cu(II). Spectrophotometric method has been developed for the assay of metoprolol tartrate, which is based on the complexation of drug with copper(II) [Cu(II)] at pH 6.0, using Britton-Robinson buffer solution, to produce a blue adduct. The latter has a maximum absorbance at 675 nm and obeys Beer's law within the concentration range 8.5-70 μg/mL. Regression analysis of the calibration data showed a good correlation coefficient (r=0.998) with a limit of detection of 5.56 μg/mL. The suggested procedure has been successfully applied to the determination of this drug in its tablets. In addition, the spectral data and stability constant for the binuclear copper(II) complex of metoprolol tartrate ($Cu_2MPT_2Cl_2$) have been reported [6].

Two simple and selective spectrophotometric methods are described for determination of metoprolol tartar as base form metoprolol in bulk drug, and in tablets and capsules by Nabil A.F., Eman M.S. The methods are based on the molecular charge transfer complexation of metoprolol base metoprolol with Bromothymol blue (BTB) or 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The yellow and orange colored radical anions formed on dissociation, are quantitated at 413 nm (BPB method) or 457 nm (DDQ method). The assay conditions were optimized. Beer's law is obeyed in the concentration ranges 2.0-40.0 μg mL^{-1} in BPB method and 5.0-25.0 μg·mL^{-1}in DDQ method, with respective molar absorptivity values of 5.78×103 and 4.05×103 L mol^{-1}·cm^{-1}. The reaction stoichiometry in both methods was evaluated by Job's method of continuous variations and was found to be 1:1 MPT-BPB, MPT-DDQ. The developed methods were successfully applied to the determination of metoprolol in pure form and commercial tablets with good accuracy and precision. Statistical comparison of the results was performed using Student's t-test and Fratio at 95% confidence level and the results showed no significant difference between the reference and suggested methods with regard to accuracy and precision. Further, the accuracy and reliability of the methods were confirmed by recovery studies via standard addition technique [7].

Ukrainian scholars Anastasiia Donchenko and Svitlana Vasyuk suggested spectrophotometric determination of metoprolol tartrate in pure and dosage forms. This method is based on the reaction between metoprolol tartrate and 2,3-dichloro-1,4-naphthoquinone in dimethylformamide (DMF) medium to form the colored reaction product with maximum absorption at 493 nm. Optimum conditions to carry out the reaction such as concentration of reagent, temperature and heating time were carefully studied and optimized. Beer's law was performed at the concentration range of 18.00-28.00 mg/100 ml. The suggested method is valid according to the validation requirements of SPhU [8].

Turkish scientist Yilmaz B. developed determination of metoprolol in pharmaceutical preparations by zero-, first-, second- and third-order derivative spectrophotometric method. Zero-, first-, second- and third-order derivative spectrophotometry methods were developed for determination of metoprolol in pharmaceutical preparations. In zero order spectrophotometry, absorbance values were measured at 276 nm in zero order spectra of solution of metoprolol in methanol in the range of 240-310 nm. In the first derivative spectrophotometry, absorbance values were measured at 265, 278 and 285 nm. In the second derivative spectrophotometry, absorbance values were measured at 276, 279, 287 and 282 nm. In the third derivative spectrophotometry, absorbance values were measured at 275, 278 and 281 nm. Parameters such as linearity, precision, accuracy, specificity, stability, limit of detection and limit of quantitation were studied according to the International Conference on Harmonization Guide-

lines. All the methods developed were successfully applied to two tablet formulation and the results were compared statistically with each other [9].

Jadhav A.S. et al. suggested UV spectrophotometric methods for estimation of metoprolol succinate from bulk and tablet formulation in phosphate buffer 6.8. The drug obeyed the Beer's law with correlation coefficient 0.9999 and 0.9979 respectively for method I and method II. It showed absorption maxima at 223 nm and 226 nm respectively for method I and method II; in phosphate buffer 6.8. The linearity was observed between 5 and 25 µg/mL. The results of analysis were validated by recovery studies, accuracy, precision, LOD, LOQ and ruggedness. The method was found to be simple, accurate, precise, economical and robust [10].

Rahman N. et al. have developed validated kinetic spectrophotometric method for determination of metoprolol tartrate in pharmaceutical formulations. The method is based on reaction of the drug with alkaline potassium permanganate at 25+/-1 degrees C. The reaction is followed spectrophotometrically by measuring the change in absorbance at 610 nm as a function of time. The initial rate and fixed time (at 15.0 min) methods are utilized for constructing the calibration graphs to determine the concentration of the drug. Both the calibration graphs are linear in the concentration range of 1.46×10^{-6}-8.76×10^{-6} M (10.0-60.0 microg per 10 ml). The calibration data resulted in the linear regression equations of log (rate)=3.634+ 0.999 log C and A=6.300 x 10^{-4}+6.491×10^{-2} C for initial-rate and fixed time methods, respectively. The limits of quantitation for initial rate and fixed time methods are 0.04 and 0.10 microg ml^{-1}, respectively. The activation parameters such as E(a), DeltaH(double dagger), DeltaS(double dagger) and DeltaG(double dagger) are also evaluated for the reaction and found to be 90.73 kJ$\epsilon$$mol^{-1}$, 88.20 kJ mol^{-1}, 84.54 $J\cdot K^{-1}\cdot mol^{-1}$ and 63.01 $kJ\cdot mol^{-1}$, respectively. The results are validated statistically and through recovery studies. The method has been successfully applied to the determination of metoprolol tartrate in pharmaceutical formulations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision [11].

The HPLC method is widely used in the analysis of metoprolol both in substance and in mono- and combined drugs. Simple, precise, sensitive methods are developed today: liquid chromatography, combination of TLC with densitometric determination, HPLC/UV, HPLC/DMD, HPLC/MS. A reverse phase HPLC method has been developed for the quantitative estimation of amlodipine besylate and metoprolol tartrate in tablet by Hussain S. et al. The quantification was carried out using RP stainless steel column ODS C18 250×4.6× 5 µ L1 packing in isocratic mode with mobile phase containing 0.03 M phosphate buffer and acetonitrile in the ratio of 32: 68 (pH 3.5). Flow rate of 1.2 ml/min and the detection wavelength were set at 230 nm and the linearity was found to be in the range of 8-12 ìg/ml for amlodipine besylate and metoprolol tartrate. The suggested method was found to be simple, precise, accurate, and reproducible for the estimation of amlodipine besylate and metoprolol tartrate [12].

Singh Brijesh et al. suggested Reverse-Phase HPLC method for simultaneous analysis of metoprolol succinate and hydrochlorothiazide in a tablet formulation. The drugs were analyzed by a reverse phase C-18 column using 50 mM di-sodium hydrogen phosphate:methanol:acetonitrile in a ratio of 525:225:250 as mobile phase. The flow rate was 1 ml/min and the compounds were detected by a UV-detector at 222 nm at a column temperature of 24±2 °C. The method was statistically validated for linearity and accuracy. The retention time and drug content of metoprolol succinate and hydrochlorothiazide were 5.38 min, 96.05% and 3.04 min., 97.64%, respectively [13].

Simultaneous estimation of metoprolol tartrate and chlorthalidone by using RP-HPLC and method development as per ICH guidelines has been suggested by P. Hari Prasad et al. The chromatographic analysis was performed on a C18column grace smart RP18 (250×4.6 mm, 5 µm) in isocratic mode, the mobile phase consisted of methanol, acetonitrile and 0.05 M phosphate buffer (adjusted topH 4.5 with orthophosphoric acid) at a ratio of 60:20:20 v/v/v, and a flow rate of 1.0 mL/min and the ASPD detector was used. The eluents were monitored at 254 nm. The retention time of lamivudine and stavudine were found to be 2.50 min and 4.25 min, respectively. The linear ranges were found to be 10-602 2 µg/mL (r=0.9992) for lamivudine and 10-60 µg/mL (r=0.999) for stavudine. The suggested method is also found to be accurate, precise and robust. The method could be applied to routine quality control of pharmaceutical formulations containing metoprolol tartrate and chlorthalidone [14].

A simple, rapid, accurate, precise, selective, and reproducible stability-indicating HPLC method has been developed for simultaneous estimation of metoprolol succinate and telmisartan using a mobile phase consisting mixture of Methanol: 10 mM potassium dihydrogen phosphate buffer: 10 mM hexane sulphonic acid (80:10:10,v/v/v) at the flow rate of 1 mL/min and detection wavelength at 223.0 nm by S. P. Mahaparale et al.. HiQ Sil C_{18} (250×4.6 mm, 5 µm) column was used as stationary phase. The retention time for metoprolol succinate and telmisartan were 3.067 min and 5.653 min, respectively. Linearity was observed in the concentration range of 5-80 µg/mL for metoprolol succinate and 5-60 µg/mL for telmisartan. The coefficient of correlation for metoprolol succinate and telmisartan was found to be 0.9990 and 0.9980, respectively. The results of analysis have been validated statistically and by recovery studies. Both the drugs were subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, and photolytic degradation. The degradation products of telmisartan and metoprolol succinate were well resolved from the pure drugs with significant differences in the retention time values. This method can be successfully implemented for simultaneous quantitative analysis of metoprolol succinate and telmisartan in bulk drugs and formulations [15].

Braza A.J. et al. suggested development, validation and analytical error function of two chromatographic methods with fluorometric detection for determination of bisoprolol and metoprolol in human plasma, which describes two high-performance liquid chromatographic methods for the individual determination of bisoprolol and metoprolol in human plasma. Analytical methods involve two different liquid-liquid extractions of human plasma, with diethyl ether for bisoprolol and with dichloromethane for metoprolol, coupled with a similar Nucleosil C(18) reversed-phase HPLC column. Fluorimetric detection was used to identify both beta-blockers. Retention times for bisoprolol and metoprolol were 8.7 and 3.2 min, respectively. Linear regressions for the calibration curves were linear at a concentration range of 6.25-200 ng/mL. Intra- and inter-day precision coefficients of variations and accuracy bias were acceptable (within 15%) over the entire range for both drugs. Average recovery was 89% for metoprolol and 98% for bisoprolol. Once the methods had been validated, analytical error functions were established as standard deviation (SD; ng/mL)=$2.216+3.608\times10^{-4}C^2$ (C=theoretical concentration value) and SD-(ng/mL)= $0.408+0.378\times10^{-1}C$ for bisoprolol and metoprolol, respectively. The developed methods and their associated analytical error functions would be suitable for pharmacokinetic studies and for determination of plasma concentration if posology individualization of these drugs is needed [16].

Chiu F.C. et al. have developed efficient high-performance liquid chromatographic assay for the simultaneous determination of metoprolol and two main metabolites in human urine by solid-phase extraction and fluorescence detection. The simultaneous analysis of the zwitterionic metoprolol acidic metabolite (III, H117/04) with the basic metabolites alpha-hydroxymetoprolol (II, H119/66), metoprolol (I) and guanoxan (IV, internal standard) was achieved employing solid-phase extraction and isocratic reversed-phase HPLC. The analytes were extracted from urine (100 microliters) using C18 solid-phase extraction cartridges (100 mg), and eluted with aqueous acetic acid (0.1%, v/v)-methanol mixture (40:60, v/v, 1.2 ml). The eluents were concentrated (250 microliters) under vacuum, and aliquots (100 microliters) were analyzed by HPLC with fluorescence detection at 229 nm (excitation) and 309 nm (emission) using simple isocratic reversed-phase HPLC (Novapak C18 radial compression cartridge, 4 microns, 100×5 mm I.D.). Acetonitrile-methanol-TEA/phosphate buffer pH 3.0 (9:1:90, v/v) was employed as the eluent (1.4 ml/min). All components were fully resolved within 18 min, and the calibration curves for the individual analytes were linear (r_2≥0.996) within the concentration range of 0.25-40.0 mg/ml. Recoveries for all four analytes were greater than 76% (n=4). The assay method was validated with intra-day and inter-day variations less than 2.5% [17].

Scientists Johnson R.D. and Lewis R.J. have developed a liquid chromatography with mass spectrometric detection (LC/MS) method for the simultaneous quantitation of three commonly prescribed beta-blockers, atenolol, metoprolol and propranolol. One advantage of the LC/MS method is the specificity provided by an ion trap MS. Utilizing an ion trap MS were able to conduct MS/MS and MS/MS/MS on each analyte. This method also eliminates the time-consuming and costly derivitization step necessary during GC/MS analysis. Additionally, by utilizing this novel method, any concerns about beta-blocker metabolite and/or sample matrix

interference are eliminated. The limits of detection for this method ranged from 0.39 to 0.78 ng/mL and the linear dynamic range was generally 1.6-3200 ng/mL. The extraction efficiencies for each analyte ranged from 58% to 82%. This method was successfully applied to postmortem fluid and tissue specimens obtained from victims of three separate aviation accidents [18].

A stereoselective liquid chromatography-tandem mass spectrometry assay was developed and validated for quantification of S- and R-metoprolol at concentrations of 0.5-50 microg/L in human plasma by Jensen B.P et al. Metoprolol was extracted from plasma by liquid-liquid extraction with ethyl acetate (82% recovery). Chromatographic separation of the enantiomers was achieved on a chiral Chirobiotic T column using an isocratic mobile phase consisting of methanol/acetic acid/ammonia (100/0.15/0.15, v/v/v). An ion trap mass spectrometer with an electrospray interface was used for detection in the positive mode, monitoring the m/z transition 268-->191 for metoprolol. Standard curves for S- and R-metoprolol fitted quadratic functions (r(2)>or=0.9995) over the range of 0.5-50 microg/L in plasma, with 0.5 microg/L representing the limit of quantification. In this range, relative standard deviations were <6% for intra-day precision and <10% for inter-day precision. The accuracy was within the range of 92-105% [19].

A simple, rapid, sensitive and specific liquid chromatography-tandem mass spectrometry method has been developed and validated for quantification of metoprolol tartrate and ramipril in human plasma by Gowda K.V. et al. Both the drugs were extracted by liquid-liquid extraction with diethyl ether-dichloromethane (70:30, v/v). The chromatographic separation was performed on a reversed-phase C8 column with a mobile phase of 10 mM ammonium formate-methanol (3:97, v/v). The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer. The method was validated over the concentration range of 5-500 ng/ml for metoprolol and ramipril in human plasma. The precursor to product ion transitions of m/z 268.0-103.10 and m/z 417.20-117.20 were used to measure metoprolol and ramipril, respectively [20].

Albers S. et al. have developed HPLC quantification of metoprolol with solid-phase extraction for the drug monitoring of pediatric patients. Chromatographic analysis was performed on a Spherisorb C(6) column (5 microm particle size) at ambient temperature and fluorimetric detection with an excitation wavelength of 225 nm, and emission wavelength of 310 nm. The mobile phase [30% acetonitrile and 70% 0.25 m potassium acetate buffer (pH 4)] was pumped with 1 mL/min. Metoprolol recovery was determined at 73.0 +/- 20.5%, and the limit of quantitation was 2.4 ng/mL. Precision values of intra- and inter-assay were below 15.5% and those for accuracy were between 90 and 110%. This method was developed for monitoring and determination of pharmacokinetic parameters of metoprolol in pediatric patients and therefore metoprolol plasma concentrations in a 2-year-old child with ventricular tachycardia were reported [21].

Angier M.K. et al. suggested gas chromatographic-mass spectrometric differentiation of atenolol, metoprolol, propranolol, and an interfering metabolite product of metoprolol. Atenolol, metoprolol, and/or propranolol, with their possible metabolite(s), were re-extracted from the selected case specimens, derivatized with pentafluoropropionic anhydride (PFPA), and analyzed by gas chromatography-mass spectrometry (GC-MS). The MS spectra of these three antihypertensives and a metoprolol metabolite were nearly identical. All of the PFPA derivatives had baseline GC separation, with the exception of a metoprolol metabolite product, which co-eluted with atenolol. There were four primary mass fragments (m/z 408, 366, 202, and 176) found with all of the PFPA-beta-blockers and with the interfering metabolite product. However, atenolol had three unique fragments (m/z 244, 172, and 132), metoprolol had two unique fragments (m/z 559 and 107), propranolol had four unique fragments (m/z 551, 183, 144, and 127), and the metoprolol metabolite product had two unique fragments (m/z 557 and 149). These distinctive fragments were further validated by using a computer program that predicts logical mass fragments and performing GC-MS of deuterated PFPA-atenolol and PFPA-propranolol and of the PFPA-alpha-hydroxy metabolite of metoprolol. By using the unique mass fragments, none of the pilot fatality cases were found to contain more than one beta-blocker. Therefore, these mass ions can be used for differentiating and simultaneously analyzing these structurally similar beta-blockers in biological samples [22-24].

From the above-mentioned, chromatographic methods of analysis amongst others

have the greatest specificity and objectivity and allow qualitative and quantitative determination of API in combinate dosage forms and biological fluids without prior separation of the components. We can conclude that analysts are constantly working on developing new methods of analysis and their optimization in order to save time and consumables, which also ensures the efficiency of the developed method. The main disadvantage of the described methods of API analysis can be considered long term from the beginning of chromatography to API release and specific solvents used as the mobile phase in HPLC. It is necessary to develop methods and to select such chromatographic conditions that provide high speed and high efficiency at lower pressure of the system. This reduces the amount of used mobile phase, which reduces cost analysis accordingly, while at the same time providing the necessary specificity, accuracy and reproducibility of the results of the analysis during quality control. Also, the reduction of analysis time is achieved by simplifying the conditions for sample preparation.

Conclusions

Thus, analysts are constantly working on developing new methods of analysis and their optimization in order to save time and consumables, which also ensures efficiency of the developed method. There is no monograph on the substance or dosage forms of metoprolol in SPhU. Therefore, it would be appropriate to recommend some of the developed methods for the SPhU monograph, which is important for ensuring pharmacopeial quality control of medicines in Ukraine.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Horyn M.M. – formal analysis, investigation, writing – original draft, *Logoyda L.S.* –conceptualization, supervision, writing – review & editing.

МЕТОДИ АНАЛІЗУ МЕТОПРОЛОЛУ У ЛІКАРСЬКИХ ЗАСОБАХ ТА БІОЛОГІЧНИХ РІДИНАХ: ОГЛЯД ЛІТЕРАТУРИ ТА МОЖЛИВІ ШЛЯХИ ЗАСТОСУВАННЯ

М.М. Горин, Л.С. Логойда

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Розробка аналітичних методів все більше впроваджується у основні практики фармацевтичної хімії та фармацевтичного аналізу з урахуванням їх високої чутливості, точності, специфічності та відтворюваності.*

Мета роботи. *Критеріями пошуку була розробка аналітичного методу метопрололу.*

Методи. *Огляд літератури було проведено упродовж 1990–2019 років, щоб зробити огляд оновленим та всеосяжним та показати нові підходи до розробки методів аналізу метопрололу. Джерелами були визнані всесвітньо журнали, а ключовими словами, які використовувались як фільтр, були метопролол, спектрофотометрія, високоефективна рідинна хроматографія, кількісний аналіз, розробка методів, валідація.*

Результати. *Хроматографічні методи аналізу, серед інших, мають найбільшу специфіку та об'єктивність та дозволяють якісно та кількісно визначити активний фармацевтичний інгредієнт (АФІ) в комбінованих лікарських формах та біологічних рідинах без попереднього розділення компонентів. Можна зробити висновок, що аналітики постійно працюють над розробкою нових методів аналізу та їх оптимізацією з метою економії часу та витратних матеріалів, що також забезпечує ефективність розробленого методу. Основним недоліком описаних методів аналізу АФІ можна вважати тривалий час від початку хроматографування до виходу АФІ та специфічні розчинники, що використовуються як мобільна фаза в ВЕРХ. Необхідно розробити методи та підібрати такі хроматографічні умови, які забезпечать високу швидкість та високу ефективність при нижчому тиску системи. Це зменшує кількість використовуваної мобільної фази, що відповідно зменшує аналіз витрат, одночасно забезпечуючи необхідну специфічність, точність та відтворюваність результатів аналізу під час контролю якості. Також скорочення часу аналізу досягається шляхом спрощення умов підготовки проби.*

Висновки. *Можна зробити висновок, що хіміки-аналітики постійно працюють над розробкою нових методик аналізу та їх оптимізацією з метою економії часу та витратних матеріалів, що також*

забезпечує ефективність розробленого методу. На даний час не має монографії на субстанцію або лікарські форми метопрололу в ДФУ. Тому було б доцільно рекомендувати деякі розроблені методики для монографії ДФУ, що є важливим для забезпечення фармакопейного контролю якості лікарських засобів в Україні.

КЛЮЧОВІ СЛОВА: **метопролол; спектрофотометрія; високоефективна рідинна хроматографія; кількісний аналіз; розробка методу; валідація.**

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QSAR-ANALYSIS OF POLYSUBSTITUTED FUNCTIONALIZED AMINOTHIAZOLES WITH ANTIHYPERTENSIVE ACTIVITY

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Background. *QSAR analysis is an important tool for the identification of pharmacophore fragments in biologically active substances and helps optimize the search for new effective drugs.*

Objective. *The aim of the study was to determine the molecular descriptors for QSAR analysis of polysubstituted functionalized aminothiazoles as a theoretical basis for purposeful search de novo of potential antihypertensive drugs among the investigated compounds.*

Methods. *Calculation of molecular descriptors and QSAR-models creation was carried out using the HyperChem 7.5 and BuildQSAR packages.*

Results. *The calculation of a number of molecular descriptors (electronic, steric, geometric, energy) was performed for 15 new polysubstituted functionalized aminothiazoles, with established in vivo antihypertensive activity. According to the calculated molecular descriptors and antihypertensive activity parameter, the QSAR models were derived HA = a + b · X1 + c · X2 + d · X3 , where the activity parameter HA is antihypertensive activity and X1, X2, X3 are molecular descriptors.*

Conclusion. *The study of 'the structure - antihypertensive activity' relationship for polysubstituted functionalized aminothiazoles was carried out. QSAR analysis revealed that volume, area, lipophilicity, dipole moment, refractivity, polarization of the molecule and energy of the lowest unoccupied molecular orbital have the most significant effect on antihypertensive activity. It was suggested that the attained QSAR-models may have antihypertensive activity within abovementioned row of compounds and can be considered as theoretical basis for de novo design of new potential antihypertensive drugs.*

KEY WORDS: **polysubstituted functionalized aminothiazoles; antihypertensive activity; molecular descriptors; QSAR-analysis.**

Introduction

QSAR analysis is an important tool for identification of pharmacophore fragments in biologically active substances and helps optimize the search for new effective drugs. To date, systematic correlation analysis is a necessary step for lead-compounds identification in the search for a new drug. The directed search for new biologically active substances can be succeeded by the use of virtual methods of research, which are carried out by means of QSAR/QSRR analysis (quantitative structure-activity/quantitative structure-property relationship), which allows establishing quantitative patterns of relation between the activity or properties of the investigated compounds and the parameters of their molecular structure. Quantitative Structure - Activity Relationship is nowadays widely used as a method for predicting the biological activity of new compounds. [1-3].

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Thiazole derivatives are a promising class for the search for biologically active compounds, since the thiazole nucleus is a strong biophore fragment for the rational design of 'drug-like molecules', and thiazole derivatives exhibit various types of biological activity: anti-inflammatory [4], antihypertensive [5], cardioprotective [6], antioxidant [7].

The **objectieve** of the study was to determine the molecular descriptors for QSAR analysis of the new polysubstituted functionalized aminothiazoles as a theoretical basis for search for new antihypertensive drugs among abovementioned types of compounds.

Methods

15 polysubstituted functionalized aminothiazoles, which antihypertensive activity was determined, were used [6, 8-9]. Calculation of molecular descriptors was carried out using Hyper-Chem 7.5 software [10] (license on HyperChem 7.5 software is available for Danylo Halytsky Lviv National Medical University); BuildQSAR software was used for QSAR-model building [11].

Results

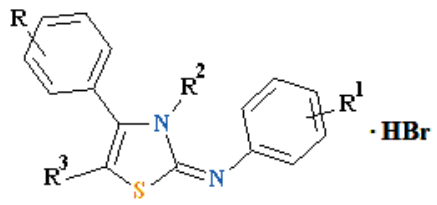
Target polysubstituted functionalized aminothiazoles were synthesized as described [6, 8-9]. The structures of the test compounds are presented in Fig. 1.

Antihypertensive activity (HA) studies were performed *in vivo* experiments on laboratory white rats [9]. The activity of the test compounds was determined by a decrease in the blood pressure in the tail artery of the animal in 60 minutes after intraperitoneal injection. For screening studies of antihypertensive activity, substances at a dose of 7 mg/kg were used. The antihypertensive activity of the test compounds are presented in Table 1.

The 2D structures of the molecules of the test compounds were converted into 3D using Hyper-Chem 7.5 software [10]. The optimization of the structures of the tested substances was carried out by the method of molecular mechanics MM+, and the final energy minimization was carried out by semi-empirical quantum-chemical AM1 method until a standard deviation of 0.001 kcal/mol was achieved. The calculation of number of descriptors (electronic, steric, geometric, energy) [12,13] was carried out for the tested compounds. The molecular descriptors were calculated using Hyper-Chem 7.5: the surface and grid area of the molecule (SA and SG), volume of the molecule (V), lipophilicity parameter (logP), refractivity (R), polarizability (P), molecular weight (M), dipole moment (D). The parameters of the calculated molecular descriptors of the test compounds are presented in Table. 1.

The following parameters were also calculated for Oxygen (Ch_O), Sulfur (Ch_S), Nitrogen of thiazole cycle (Ch_Nt) and Nitrogen of amino-group (Ch_N); distances between atoms of Sulfur and Oxygen (D_S-O), Sulfur and Nitrogen Nt (D_S-Nt), Nitrogen of the amino-group and Nitrogen of the thiazole cycle (D_Nt-N); angles between the atoms of the Nitrogen of the thiazole cycle, Sulfur and Nitrogen of the amino group (A_N-S-Nt), Nitrogen of the amino group, Oxygen and Nitrogen of the thiazole cycle (A_N-O-Nt). The attained parameters are presented in Table 2.

The energy parameters of the studied molecules were assessed: total energy (TE), binding energy (BE), isolated atomic energy (IAE), electronic energy (EE), core-core interaction (CCI), heat of formation (HF), energies of the highest occupied molecular orbital and lowest unoccupied molecular orbital (HOMO and LUMO), hydration energy (EH). The attained results of energy parameters are presented in Table 3.



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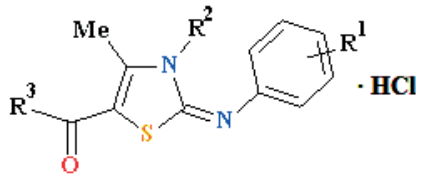

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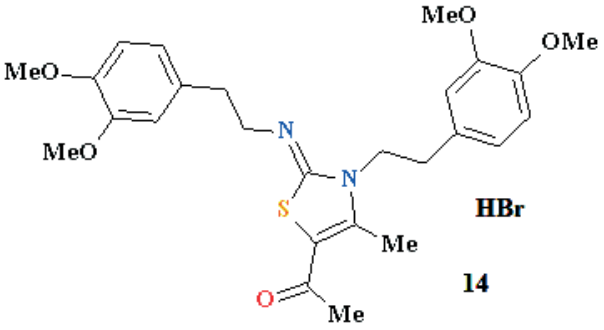

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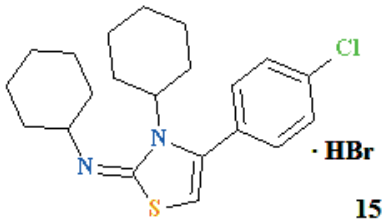

15

1. **R**= 4-OMe, **R¹**=2,5-diMe, **R²**= $(CH_2)_2OH$, **R³**=H
2. **R**= 4-OMe, **R¹**=4-$OCHF_2$, **R²**= $(CH_2)_3OH$, **R³**=Me
3. **R**= 4-$OCHF_2$, **R¹**=4-OMe, **R²**= $(CH_2)_2OH$, **R³**=H
4. **R¹**= H, **R²** = N-piperydinyl$(CH_2)_2$, **R³**=H,
5. **R¹**= 4-OMe, **R²**= N-morpholinyl$(CH_2)_2$-, **R³**=OEt,
6. **R¹**= 2,3-diMe, **R²** = $(CH_2)_2OH$, **R³**=OEt,
7. **R¹**= 4-Me, **R²**= 3-pyridinyl-CH_2, **R³**=H,
8. **R¹**= 4-Me, **R²**= N-morpholinyl, **R³**=H,
9. **R¹**= 4-OEt, **R²**= N-morpholinyl, **R³**=H,
10. **R¹**= 4-Me, **R²**= 3,4-diOMe-$C_6H_3(CH_2)_2$, **R³**=H,
11. **R¹**= H, **R²**= 3,4-diOMe-$C_6H_3(CH_2)_2$, **R³**=H,
12. **R¹**= 2-Me, **R²**= 3,4-diOMe-$C_6H_3(CH_2)_2$, **R³**=H,
13. **R¹**= 2,3-diMe, **R²**= 3,4-diOMe-$C_6H_3(CH_2)_2$, **R³**=H,

Fig. 1. Structures of the tested compounds.

Table 1. Antihypertensive activity and molecular descriptors of polysubstituted functionalized aminothiazoles

No.	AH	SA	SG	V	logP	R	P	M	D
1	8	546.8	667.17	1142.6	1.3	122.22	43.77	435.38	1.99
2	11.9	705.14	731.37	1248.5	0.93	130.69	46.06	501.39	7.11
3	10.5	642.6	666.28	1141.6	0.85	120.04	42.39	473.33	4.49
4	6.2	551.29	605.91	1063.4	1.22	113.24	41.49	380.94	3.29
5	14,3	666.39	680.82	1224.9	1.7	124.63	45.78	470.42	7.44
6	5.5	514.36	560.73	969.72	0.68	99.07	36.3	334.43	4.21
7	8.3	511.78	577	981.94	0.68	107.71	38.47	337.44	2.2
8	6.6	570.15	579.33	1015.7	0.68	106.46	38.94	367.89	2.83
9	8.2	618.67	628.25	1094.2	-0.12	113.31	41.41	397.92	3.07
10	7.8	588.77	639.32	1147.8	0.42	128.78	45.96	410.53	2.04
11	12.2	549.76	614.72	1100.6	0.27	124.5	44.1	396.5	2.24
12	2.1	567.3	629.3	1137.4	0.42	106.94	42.56	410	2.49
13	11.1	659	672.16	1266	1.36	142.63	51.19	505.47	3.97
14	9.2	692.98	743.14	1354	-0.94	146.17	52.54	484.86	3.72
15	10.8	530.62	639.61	1153.6	4.88	123.15	46.06	455.88	2.28

Table 2. Charges, distances, angles between atoms of polysubstituted functionalized aminothiazoles

No.	Ch_O	Ch_S	Ch_Nt	Ch_N	D_S-O	D_S-Nt	D_S-N	D_Nt-N	A_N-S-Nt	A_N-O-Nt
1	0	0.382	-0.204	-0.243	0	2.59	2.79	2.37	52.27	0
2	0	0.285	-0.227	-0.205	0	2.60	2.79	2.38	52.41	0
3	0	0.352	-0.215	-0.229	0	2.59	2.79	2.38	52.31	0
4	-0.281	0.392	-0.204	-0.24	3.53	2.59	2.79	2.37	52.32	21.86
5	-0.363	0.449	-0.227	-0.207	2.6	2.61	2.63	2.49	56.86	22.08
6	-0.354	0.489	-0.243	-0.214	3.37	2.60	2.63	2.50	56.93	23.3
7	-0.271	0.384	-0.209	-0.223	3.34	2.59	2.79	2.38	52.42	22.16
8	-0.268	0.366	-0.117	-0.191	3.42	2.63	2.77	2.41	51.79	32.96
9	-0.261	0.374	-0.125	-0.193	3.27	2.62	2.77	2.41	53.1	23.14
10	-0.287	0.405	-0.203	-0.248	3.16	2.59	2.78	2.38	52.5	23
11	-0.286	0.406	-0.202	-0.25	3.17	2.59	2.78	2.38	52.53	23.04
12	-0.285	0.405	-0.203	-0.251	3.18	2.59	2.78	2.38	52.4	22.85
13	-0.286	0.361	-0.206	-0.238	3.13	2.59	2.78	2.38	52.37	23.13
14	-0.282	0.356	-0.211	-0.273	3.23	2.6	2.76	2.4	53	22.94
15	0	0.292	-0.183	-0.251	0	2.64	2.76	2.41	52.97	0

In the study, the optimal set of molecular descriptors was chosen by a sequential algorithm and the Multiple Linear Regression (MLR) method to obtain QSAR models by means of BuildQSAR [11]. Statistically, the number of compounds tested (N) and independent variables (M) used in the model should correspond to a ratio of N / M ≥ 5. Descriptors with high pairwise correlation were excluded from the multidimensional descriptor space. Firstly, the descriptors from different groups were used to build individual models, and then descriptors that most fully describe the change in biological activity were used to obtain mixed models. This allowed us to choose single or multi-parameter models with the maximal correlation coefficient (r) and the minimal standard deviation (s). The models were further investigated for their adequacy by means of the Fisher coefficient (F) and predictive ability using the Q^2 cross-validation coefficient and sum of squares of prediction error (SRRESS).

Based on the calculated molecular descriptors and the parameter of antihypertensive activity, the QSAR models were derived **AH=a+b·X1+c·X2+d·X3** , where the activity parameter AH is antihypertensive activity and X1, X2, X3 are molecular descriptors:

Table 3. Energy parameters of polysubstituted functionalized aminothiazoles

No.	TE	BE	IAE	EE	CCI	HF	HOMO	LUMO	EH
1	-103278	-5050	-98228	-780638	677360	4.33	-7.81	-0.27	-5.71
2	-136024	-5476	-130548	-989792	853768	-153.1	-8.12	-0.56	-12.2
3	-128838	-4913.8	-123924	-872337	743499	-141.4	-7.92	-0.59	-11.9
4	-100651	-4965.6	-95685	-785689	685038	19.91	-7.97	-0.34	-3.82
5	-117058	-5558.6	-111499	-960851	843794	-71.13	-8.34	-0.48	-2.35
6	-93372	-4600.7	-88771	-679708	586336	-78.24	-8.26	-0.42	-5.84
7	-88276	-4631.9	-83644	-648490	560214	69.86	-8.01	-0.41	-3.63
8	-99334	-4601.6	-94733	-733476	634141	3.26	-8.22	-0.45	-2.42
9	-110306	-4971.6	-105334	-827964	717658	-32.06	-8.05	-0.45	-4.36
10	-112313	-5773	-106539	-940854	828541	-17.24	-7.93	-0.48	-3.82
11	-108718	-5490	-103228	-889289	780571	-9.39	-8.03	-0.489	-5.25
12	-112312	-5772	-106539	-952370	840058	-16.28	-7.95	-0.46	-4.33
13	-124384	-6144	-118239	-1097703	973319	-34.41	-8.12	-0.65	-3.25
14	-137848	-6793	-131054	-1237984	1100137	-92.91	-8.19	-0.21	-6.53
15	-101712	-5379	-96332	-842684	740972	15.79	-8.21	-0.31	0.58

HA=-0.044(±0.019)**SA**+0.041(±0.011)**V**-24.062(±7.933)**LUMO**-21.062(±8.297) **(1)**

(n=015; r=0.936; s=1.171; F=26.006; Q^2=0.799; SPRESS=1.492)

HA=+0.048(±0.015)**V**+0.004(±0.001)**IAE**-27.025(±9.356)**LUMO**-29.957(±10.172) **(2)**

(n=015; r=0.930; s=1.227; F=23.353; Q^2=0.718; SPRESS=1.767)

HA=+0.019(±0.011)**V**+0.731(±0.866)**logP**-13.142(±9.671)**LUMO**-19.284(±13.242) **(3)**

(n=015; r=0.825; s=1.879; F=7.844; Q^2=0.500; SPRESS=2.354)

HA=+0.051(±0.028)**R**-0.002(±0.001)**BE**-17.152(±10.844)**LUMO** -16.857(±13.668) **(4)**

(n=015; r=0.813; s=1.940; F=7.132; Q^2=0.086; SPRESS=3.470)

HA=+0.033(±0.026)**R**+0.423(±0.304)**P**-17.522(±10.543)**LUMO** -20.150(±14.952) **(5)**

(n=015; r=0.825; s=1.880; F=7.840; Q^2=0.295; SPRESS=2.795)

HA=+1.237(±0.685)**P**+0.002(±0.001)**EE**-22.339(±12.913)**LUMO**-36.725 (±21.841) **(6)**

(n=015; r=0.817; s=1.920; F=7.359; Q^2=0.313; SPRESS=2.760)

HA=+1.165(±0.669)**P**-0.002(±0.001)**CCI**-20.886(±12.661)**LUMO**-35.268(±21.945) **(7)**

(n=015; r=0.807; s=1.968; F=6.835; Q^2=0.296; SPRESS=2.793)

HA=+0.019(±0.012)**V**-11.777(±10.599)**LUMO**+0.275(±0.771)**A_N-S-Nt**-32.425(±42.963) **(8)**

(n=015; r=0.778; s=2.092; F=5.624; Q^2=0.115; SPRESS=3.132)

HA=+0.019(±0.012)**V**-11.197(±11.685)**LUMO**+6.871(±57.283)**Ch_N**-16.433(±20.007) **(9)**

(n=015; r=0.766; s=2.142; F=5.196; Q^2=0.158; SPRESS=3.055)

HA=+0.526(±0.373)**P**-12.807(±11.969)**LUMO**+19.091(±33.934)**D_N-Nt**-65.167(±86.579) **(10)**

(n=015; r=0.721; s=2.307; F=3.973; Q^2=0.028; SPRESS=3.284)

HA=+0.023(±0.012)**V+**0.233(±0.391)**EH-**14.248(±10.989)**LUMO**-21.927(±15.461) **(11)**

(n=015; r=0.799; s=2.001; F=6.492; Q^2=0.351; SPRESS=2.683)

HA=+0.012(±0.015)**V**+0.535(±1.072)**logP**+0.528(±0.934)**D**-6.815(±14.912) **(12)**

(n=015; r=0.705; s=2.362; F=3.622; Q^2=0.048; SPRESS=3.409)

Discussion

According to the analysis of the derived QSAR models **(1-12)**, it was established that the antihypertensive activity increases with the increase of the parameters of the following molecular descriptors: volume, lipophilicity, dipole moment, refractivity and polarization of the molecule and decrease of the surface area of the molecule. Among the energy parameters, the most significant effect is the energy of the lowest unoccupied molecular orbital, with increasing antihypertensive activity and decrease in this parameter. Antihypertensive activity also increases with increasing charge on the Nitrogen atom of the amino group, with increasing distance between this atom and the Nitrogen atom of the thiazole cycle and increase of the angle A_N-S-Nt**.**

The dependence of the observed and predicted antihypertensive activities for the QSAR models **(1-3)** is presented in Fig. 2.

The search for quantitative regularities of the dependence of the antihypertensive activity

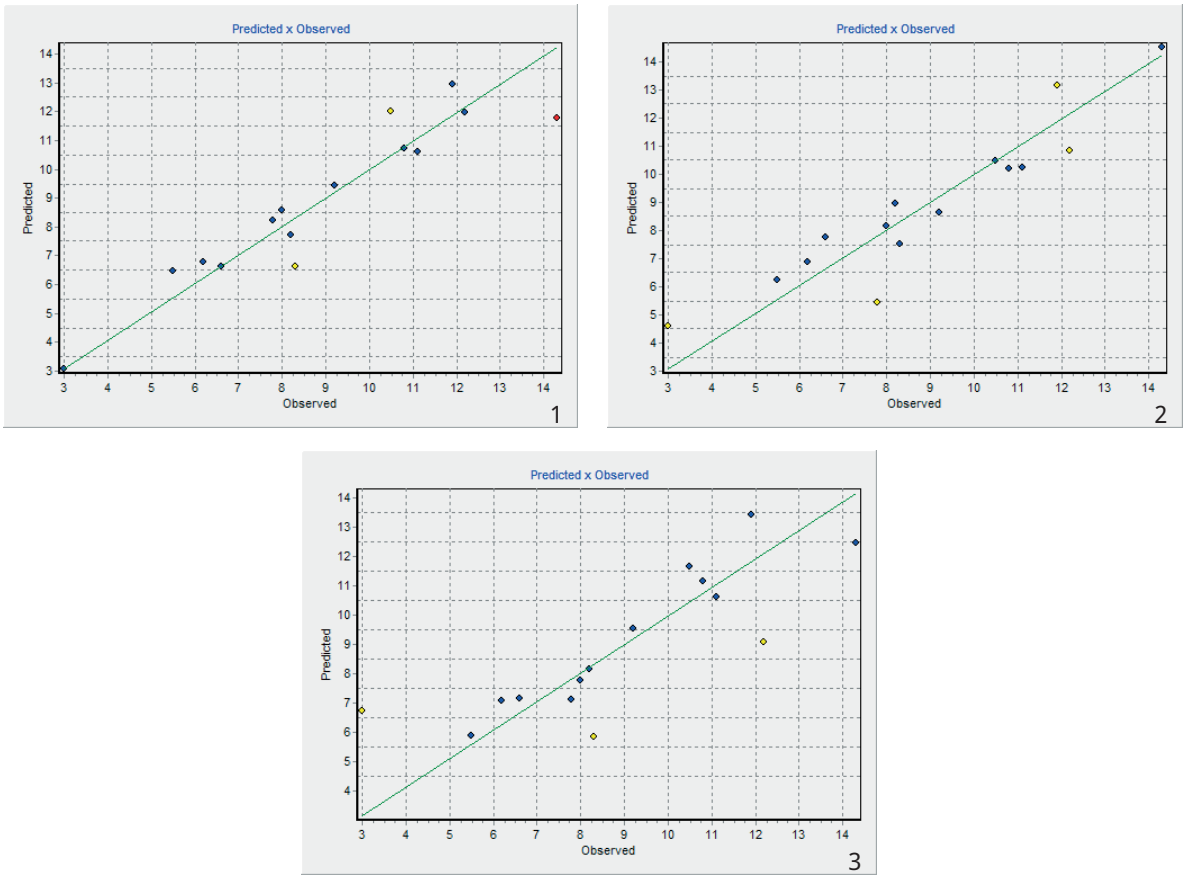

Fig. 2. The dependence of the observed and predicted antihypertensive activities for the QSAR models 1, 2, 3.

of the studied compounds on the parameters of their molecular descriptors allowed deriving statistically qualitative QSAR models **(1-3)** (r=0,825÷0,936) characterized by sufficient adequacy (F=7.8÷26.006) and predictive ability (Q^2=0.5-0.799).

Conclusions

Thus, according to the study of the 'structure-antihypertensive' activity relationship for polysubstituted functionalized aminothiazoles, the QSAR analysis revealed that the volume, area, lipophilicity, dipole moment, refractivity, polarization of the molecule and energy of the lowest unoccupied molecular orbital have the most significant effect on antihypertensive activity. The derived QSAR-models are suggested for antihypertensive activity prediction within abovementioned row of compounds and can be a theoretical basis for *de novo* development of new potential antihypertensive drugs.

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Conflict of Interests

The authors declare no conflict of interest.

QSAR-АНАЛІЗ ПОЛІЗАМІЩЕНИХ ФУНКЦІОНАЛІЗОВАНИХ АМІНОТІАЗОЛІВ З ГІПОТЕНЗИВНОЮ АКТИВНІСТЮ

І.В. Драпак

ЛЬВІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ ДАНИЛА ГАЛИЦЬКОГО, УКРАЇНА

Вступ. *QSAR-аналіз є важливим інструментом ідентифікації фармакофорних фрагментів в біологічно активних речовинах та дозволяє оптимізувати пошук нових ефективних ліків.*

Мета дослідження. *Визначення молекулярних дескрипторів для QSAR аналізу полізаміщених функціоналізованих амінотіазолів як теоретичної основи для цілеспрямованого пошуку нових антигіпертензивних засобів.*

Методи дослідження. *Розрахунок молекулярних дескрипторів та побудова QSAR-моделей проводились з використанням програмних пакетів: Hyper-Chem 7.5 та BuildQSAR.*

Результати. *Для 15 нових полізаміщених функціоналізованих амінотіазолів із встановленою in vivo гіпотензивною активністю проведено розрахунок ряду молекулярних дескрипторів (електронних, стеричних, геометричних, енергетичних). На основі розрахованих молекулярних дескрипторів та параметра гіпотензивної активності були отримані QSAR-моделі: АН = a + b · X1 + c · X2 + d · X3, де АН - гіпотензивна активність, a X1, X2, X3 - молекулярні дескриптори.*

Висновки. *Проведено дослідження зв'язку "структура – гіпотензивна активність" для ряду полізаміщених функціоналізованих амінотіазолів. За результатами QSAR-аналізу встановлено, що об'єм, площа, ліпофільність, дипольний момент, рефрактивність, поляризованість молекули та енергія нижчої незайнятої молекулярної орбіталі мають найбільш значний вплив на гіпотензивну активність. Одержані QSAR-моделі будуть використані для прогнозування активності даного ряду сполук і можуть розглядатися як теоретична основа для de novo дизайну нових потенційних гіпотензивних препаратів.*

КЛЮЧОВІ СЛОВА: **полізаміщені функціоналізовані амінотіазоли; гіпотензивна активність; молекулярні дескриптори; QSAR-аналіз.**

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ANTI-INFLAMMATORY EFFECTS OF PROPOXAZEPAM ON DIFFERENT MODELS OF INFLAMMATION

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Background. *Propoxazepam, 7-bromo-5-(2-chlorophenyl)-3-propoxy-1H-benzo[e][1,4]diazepin-2(3H)-one, is a promising analgesic and anticonvulsant and is on preclinical trial.*

Objective. *The aim of the research was to study the anti-inflammatory and analgesic action of Propoxazepam.*

Methods. *The anti-inflammatory action was evaluated by carrageenan induced rat paw edema, formalin-induced paw licking response in mice and bradykinin-induced pain response in rat models.*

Results. *It was established for the first time that the administration of Propoxazepam caused a significant anti-inflammatory activity when tested in different in vivo chemical experimental models of induced inflammation, i.e. carrageenan-, bradykinin- and formalin-induced inflammation tests.*

Conclusions. *Propoxazepam significantly reduced acute and sub-acute inflammation and proved its efficacy and similar to anti-inflammatory action.*

KEY WORDS: ***propoxazepam; diclofenac sodium; anti-inflammatory effect; carrageenan; formalin.***

Introduction

Benzodiazepines are a large and still expanding group of synthetic heterocyclic derivatives. The wide spectrum of the pharmacological effects exhibited by these compounds makes them one of the most versatile class of drugs used in psychopharmacology. Classical 1.4-benzodiazepine (BDZ) drugs have sedative, anxiolytic and anticonvulsant properties, enhancing the gamma – aminobutyric acid (GABA)-ergic neurotransmission through binding to the specific BDZ recognition sites, within GABAA receptor-ion channel complex, and allosterically modulate its activity [1]. Almost since their introduction, there has been interest to the therapeutic use of the benzodiazepines for management of pain. As regarding many other drugs, initially developed and studied for indications other than pain, conclusive data regarding the analgesic activity of BDZ is lacking. A relevant aspect of neuroplastic changes in inflammatory and neuropathic conditions is reduction in inhibitory glycinergic and GABAergic control of dorsal horn neurons: a reduction in the $GABA_A$-mediated endogenous inhibitory control within the central nervous system leads to exaggerated pain and hyperalgesia [2]. Potentiation of $GABA_A$ receptor-mediated synaptic inhibition by benzodiazepines reverses pathologically increased pain sensitivity in animal studies. Though BDZ and barbiturates have discriminating effects on pain, their pharmacological actions on the CNS are mediated through the GABA induced chloride currents [3]. In contrast to phenobarbitone, diazepam has also been reported to have anti-inflammatory action [4] and antipyretic action [5,6].

A number of 3-substituted 1.4-benzodiazepines have been synthesized at the Physico-Chemical Institute of the National Academy of Sciences of Ukraine and their structure – activity relationships, have been studied as well. Their pharmacological effect was unusual, because unlike most classical BDS, in the models of nociceptive and neuropathic pain these substances showed significant analgesic activity [7]. One of them, Propoxazepam, 7-bromo-5-(2-chlorophenyl)-3-propoxy-1H-benzo[e][1,4] diazepin-2(3H)-one, is a promising drug and is on preclinical trials [8]. Like gabapentin and pregabalin, which are well-known drugs used in general medical practice for treatment of neuropathic pain [9], Propoxazepam also has

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an anticonvulsant effect [10, 11], which explains the analgesic component of the pharmacological spectrum.

An inflammatory response and development of pain are interdependent processes. The uncontrolled inflammatory reaction triggers the process of chronic pain generation, which is associated with the phenomena of central sensitization and neuroplasticity. It becomes obvious that the main direction of complex analgesic therapy should be the targeted use of pharmacological agents possessing anti-inflammatory potential [4-6].

It has been established that acute diazepam (a BDZ derivative drug) treatment suppresses cell proliferation in rat thymus, decreases interleukin release from mouse macrophages [12] and decreases macrophage and neutrophil activities [13], most probably by affecting the peripheral-type benzodiazepine receptor (PBR) present in these immune/inflammatory cells. High doses of diazepam (10.0–20.0 mg/kg) have proved to reduce the volume of acute inflammatory paw edema in rats as a response to carrageenan administration [14]. This effect was associated with an action of diazepam on the PBR present in the adrenal and/or immune/inflammatory cells [15].

Therefore, the ***objective of the study*** was to investigate the possible anti-inflammatory effects of the innovative derivative of BDZ Propoxazepam on acute and chronic inflammatory responses in the rats exploring the possible underlying mechanisms involved in these anti-inflammatory effects.

Methods

Animals and Injection Procedures

Male Wistar rats (180-210 g in weight) and white mice (20-24 g in weight), took from Institute of Pharmacology and Toxicology of the NAMS of Ukraine, kept at the local animal facility, were used. The animals were exposed to a 12-hour light-dark cycle and were provided with food and water *ad libitum*. All experiments were conducted during the light part of the day (9.00-14.00). The experiments were carried out according to the recommendations of the Committee for Research and Ethical Issues of the IASP (1983) and were approved by the local ethical committee for animal research. All manipulations were performed to minimize animal suffering and to reduce the number of animals used.

The test compound was suspended in Tween 80 (1%) emulsion, and the control animals received corresponding amount of vehicle (1% Tween 80).

Drugs and Chemicals

Propoxazepam was synthesized according to the method described in [16]. The structure of the substance was determined and approved by a complex of physicochemical methods (IR and mass spectroscopy, as well as X-ray diffraction analysis) [17]. Chemical purity was confirmed by elemental analysis (99%). Sodium carrageenan and bradykinin were obtained from Sigma Chemical Co., USA. Diclofenac sodium salt (Merck). Various other chemicals and reagents were of analytical grade and got from local firms.

The doses of Propoxazepam for experiments on animals were chosen according to previous bioscreening and pharmacodynamic data, where the analgesic properties of Propoxazepam were estimated. For rat tests the mean effective dose (ED_{50}) in the tail flick test was 1.83 mg/kg, so in this species of animals for paw edema tests the doses were raised in 1.6 (for 3 mg/kg) and 5 times (for 10 mg/kg) in order to achieve expected 82 % (1 σ) and 95 % (2 σ) effect. Mice doses were derived according to allometric relations with regard to species and body weight coefficients.

Carrageenan-Induced Rat Paw Edema

Three groups of eight rats each were administered with the vehicle (Tween 80 (1%) emulsion, p.o.), the test compound (3 or 10 mg/kg, p.o.), and diclofenac sodium (10 mg/kg, p.o.). One hour following the treatment with various agents, edema was induced by a subplantar injection of 0.1 mL of 1% of freshly prepared suspension of carrageenan into the right hind paw of each animal. The volume of the injected paws was estimated at 0; 2 and 4 h following carrageenan injection utilizing a plethysmometer (Plethismometer, Ugo Basil, Italy). The edema development was determined by the increase in paw volume. The increase in percentage inhibition was calculated utilizing the following equations:

Percentage inhibition = $(\Delta V_к - \Delta V_д / \Delta V_к) \cdot 100\%$,

Where: $\Delta V_к$ – mean paw size in the control group.

$\Delta V_д$ – mean paw size in the treated group.

Formalin-Induced Paw Licking Response in Mice

Mice were divided into four groups (n=6). Test drugs Propoxazepam (0.01, 0.1 and 3.0 mg/kg p.o.), diclofenac sodium (10 mg/kg p.o.) and control vehicle (1% Tween 80, p.o.) were administered 1 h before formalin injection into the

animals in the first set (early phase) and 40 min before formalin injection into the animals in the second set (late phase), respectively. Mice were subcutaneously injected with 20 µl of formalin (1% in normal saline) into the right dorsal hind paw. The amount of time that the animal spent licking the injected paw was measured during the first 5 min (Phase 1, corresponding to the direct chemical stimulation of nociceptors) and 20-25 min after formalin injection (Phase 2, inflammatory).

Bradykinin-Induced pain response in rat

The possible contribution of bradykinin receptors in the antinociceptive effect of propoxazepam was evaluated by using the method described by Chau et al [18]. Bradykinin (0.01% solution) was injected to the subplantar area of rat right hind paw 0.1 ml/animal. Propoxazepam (1.83 mg/kg) was administered 2 hours prior to bradykinin injection. The degree of hyperalgesia was determined using a dolorimeter (Dolorimeter Baseline, USA) by determination of the threshold of pain sensitivity (TPS) – the minimum pressure on the lower surface of the rat's foot (g/mm^2), which caused pain in the animal (vocalization and/or withdrawal of the foot). Each animal was given 5 attempts; the threshold value was taken with such a pressure force, which caused a positive response in at least one attempt. The TPS was compared on intact and damaged limbs on the 14th day after tying (pathology without treatment), as well as on the injured limb in 2 hours (peak of action) after the drugs administration.

Data Analysis

Data are expressed as a mean and standard error mean (SEM) and a Student's t-test was used to compare the data of the control and standard groups. Probabilities (p) of <0.05 were considered statistically significant. Statistical analysis was performed using the standard statistical package of MS Excel.

Results

Effects of Propoxazepam on Acute Inflammation

A single sub-plantar injection of carrageenan induced an increase in the paw thickness within 24 hours. The rat group pretreated with Propoxazepam had a significantly reduced ($p<0.05$) increase in the paw thickness in 2 hours with 36.7%, (3.0 mg/kg) and 47.0% (10 mg/kg) reduction of the paw edema and in 4 hours – 25.0% (3.0 mg/kg) and 26.7% (10 mg/kg). While rats of the group pretreated with diclofenac sodium showed 47.0% and 26.7% ($p<0.0001$) reduction of the paw edema, respectively (Table 1).

Effect of Propoxazepam on Formalin-Induced Inflammation

As shown in Figure 1, the i.p. treatment with Propoxazepam at the doses of 0.01, 0.10 and 3.0 mg/kg significantly inhibited the licking time in of both neurogenic (0-5 min), by 91.9% ($p<0.001$), 76.0% ($p<0.001$), and 15.0%, and inflammatory (15-30 min), by 98.4% ($p<0.001$), 54.0% ($p<0.001$), and 32.1% ($p<0.01$), phases of formalin-induced paw-licking test compare to the control group. Diclofenac sodium (10,0mg/kg, i.p.) significantly inhibited both phases of the test by 51.0 % and 53.6% ($p<0.001$), respectively.

Propoxazepam Antinociceptive Action on the Bradykinin-Induced Hyperalgesia

Bradykinin injection to the rats induced statistically significant TPS decrease by 71.7% (Fig. 2). Under these conditions Propoxazepam induced prominent antibradykinin effect, since on the background of its administration bradykinin induced TPS decrease was threefold less that of in the control group (23.8% and 71.7% respectively).

Discussion

The study proves the anti-inflammatory and analgesic effect of Propoxazepam on different models of inflammation. The carrageenan-induced rat paw edema model is widely used to investigate mechanisms of inflammatory processes and to screen potential anti-inflammatory agents. This model has been extensively studied in the assessment of anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins. The edema and inflammation induced by carrageenan is established to be mediated by histamine and 5-HT during the first hour, after which increased vascular permeability is maintained by the release of kinins up to 2.30 hours and from 2.30 to 6 hours, the mediators are found to be the prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site [19, 20].

In carrageenan-induced paw edema test, the highest inhibitory activity was exhibited at the dose of 10 mg/kg compared to the other dose. This simply depicts that the higher the dose, the more the inhibition of the edema. Hence, an inhibitory activity of Propoxazepam is dose-dependent (Table 1).

The results of the study have revealed that Propoxazepam as well as diazepam has a sig-

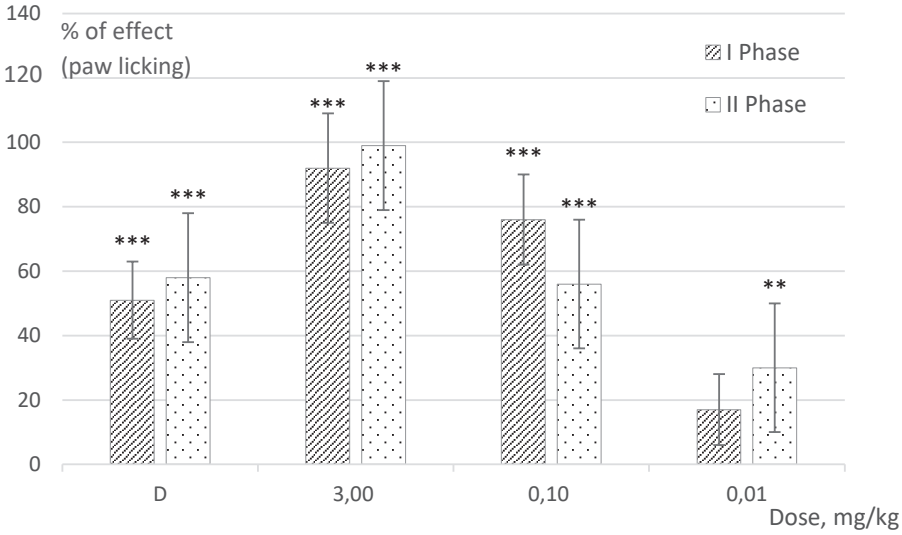

Figure 1. Effect of Propoxazepam and Diclofenac Sodium on the paw licking induced by a formalin injection in mice. Results are presented as mean±SEM (n=6).
** – p<0.01, *** – p<0.001, compared to the control.

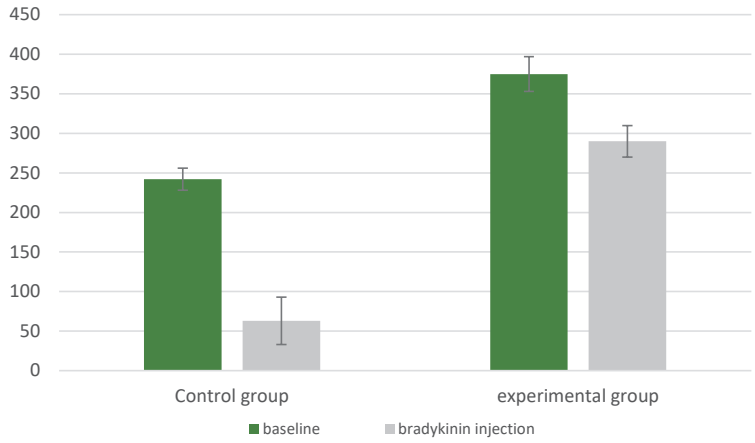

Figure 2. Effect of propoxazepam (2 mg/kg p.o.) on bradykinin-induced hyperalgesia in rats. Pressure induced hind paw withdrawal were evaluated before (baseline), and in 1 min after intraplantar bradykinin (0.01%) administration. Two hours before examination the control group received vehicle, and the experimental group received propoxazepam.
Data are presented as a mean±SEM, n=7. * – p<0.05 versus baseline, # – p<0.05 versus vehicle.

nificant anti-inflammatory effect on the different experimental models of inflammation either acute or chronic. In the acute inflammatory study, diazepam caused significant reduction of carrageenan-induced paw edema and serum NO_x levels in rats. While in the chronic inflammatory study, diazepam led to significant reduction of the paw thickness, significant reduction in the serum C-reactive protein, significant increase in serum albumin and significant increase in the serum corticosterone level [21, 22]. Similar pharmacological actions have been reported for chlordiazepoxide while, alprazolam is reported to be devoid of

Table 1. Change in paw size by carrageenan-induced paw edema in albino rats treated with increased doses of Propoxazepam

Treatment	Dose, mg/kg	Percentage inhibition (%) after 2 hours	Percentage inhibition (%) after 4 hours
Propoxazepam	3.0 10.0	36.7±4.1** 47.0±4.1***	25.00±2.7* 26.70±5.4*
Diclofenac Sodium	10.0	43.0±7.0***	57.10±8.3***

*Notes. Values are expressed as Mean±SEM of eight rats, * – p<0.05, ** – p<0.001, *** – p<0.0001 (Student's t-test).*

analgesic activity [23]. These reports clearly indicate that various benzodiazepines differ individually in their pharmacological profiles despite of the common chemical structure. Furthermore, the results of chronic inflammatory study showed that diazepam significantly increased serum albumin level and significantly decreased serum C-reactive protein, which is an acute phase protein commonly used to assess the disease activity in inflammatory rheumatic diseases, and this effect of diazepam could be explained by the fact that it can suppress secretion of pro-inflammatory cytokines from mouse macrophages such as TNF-α, IL-1, and IL-6, which is the main stimulator of acute phase protein synthesis in acute and chronic inflammation [24]. Structurally Propoxazepam is closer to diazepam and chlordiazepoxide, therefore their mechanisms of action coincide.

The formalin-induced licking response is used as a model for evaluation of new analgesic. This definitely proves whether the licking is genuinely due to formalin injected into the paw, because at times the animals lick the forepaw under normal physiological conditions. However, formalin test is sensitive to non-steroidal anti-inflammatory drugs and other mild analgesics. The test possesses two distinct phases, possibly reflecting different stages of pain. The early phase reflects a direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects inflammatory pain [25]. Moreover, formalin-induced nociception is also associated with direct action on a member of Transient Receptor Potential family (TRP) of cation channels denoted as TRPA1 receptor located in C fibers [26]. The results of the study show that the i.p. administration of Propoxazepam significantly and dose-dependently attenuates the nociceptive response in both neurogenic and inflammatory phases of the formalin-induced paw-licking test in mice at the level reference drug diclofenac sodium. The effect of diazepam is very similar to the effect of Propoxazepam in formalin-induced licking model [27]. The effect of diazepam is confined to increase the pain scores during the periods in 10, 15, and 20 min after formalin.

One of the cardinal features of inflammatory states is that normally innocuous stimuli produces pain [28]. Bradykinin, one of the peptide kinins, is an important inflammatory mediator. The main function of bradykinin is to increase the sensation of pain. A secondary function of bradykinin is to promote the production of histamine that is of increasing blood flow into the involved area by dilation of arteries and increased capillary vessel permeability [29].

Bradykinin diversely influences on the pathophysiological processes accompanying pain and inflammation. Its biological action is mediated by two established G-protein coupled receptors named B1 and B2. The bradykinin B2 receptor is constitutively expressed in most cell types and evokes acute pain responses following tissue injury, whereas the bradykinin B1 receptor is induced during inflammatory insults or painful stimuli [30].

The attained results show that Propoxazepam in this experiment reduces hyperalgesia on the in the model of bradykinin-induced edema. An additional argument for possible interaction of Propoxazepam with bradykinin receptors is the study [31] of compound influence on the maximal normalized speed of bradykinin-induced contraction of the rat stomach smooth muscles in the presence of gadolinium ions and verapamil. For Propoxazepam the statistically significant changes of the before-mentioned indicator have been shown as it is able to additionally inhibit the bradykinin-induced contraction in the presence of Gd^{3+} and verapamil by 19.3% and 32.0% respectively, and demonstrates the effects similar to those of des-Arg9-bradykinin-acetate (B2-bradykinin receptors concurrent antagonist) that proves either interaction with receptor or influence on signal transduction pathways.

Additionally, the mechanisms and antinociceptive effects of propoxazepam were studied on animal models of acute and chronic pain [7, 8]. The effects of Propoxazepam on pain responses were examined using tail-flick test (TFT) in rats, streptozotocin-induced rat model (SPZ) and sciatic nerve injury (SNI)-induced hyperalgesia in rats. Propoxazepam (3 mg/kg)) proved statistically significant analgesic effect compare to the control and ketorolac values after acute application in TFT and SNI-induced hyperalgesia in rats. Propoxazepam (2 mg/kg) compare to gabapentin (5 mg/kg) in greater degree after both single and chronic administrations showed analgesic action in SPZ-diabetic rats. Propoxazepam administration reduced bradykinin-induced (0.01 %) hyperalgesia. At a low dose (1 mg/kg) flumazenil diminished Propoxazepam antinociceptive effect while at a higher dose (10 mg/kg) had nearly no influence, possibly due to GABAA-receptor complex stabilization. This suggests

that Propoxazepam causes both nociceptive and neuropathic analgesia in rats and $GABA_A$-receptor and bradykinin B-receptor are key sites of analgesic action of propoxazepam.

Conclusions

The study established the anti-inflammation effects caused by oral administration of a novel synthetic 1,4-benzodiazepine analogue, 7-bromo-5-(2-chlorophenyl)-3-propoxy-1H-benzo[e][1,4]diazepin-2(3H)-one (Propoxazepam) into the rats and mouse models with induced inflammation and nociception and explored potential mechanisms of its action. It was proved for the first time that the administration of Propoxazepam had significant anti-inflammatory action when tested in different in vivo chemicals experimental models of induced inflammation, namely carrageenan-, bradykinin- and formalin-induced inflammationtion tests. The mechanism of Propoxazepam action might be the inhibition of synthesis or release of inflammatory mediators. As a result of the notable biological activity of Propoxazepam it would be applicable to conduct additional research to implement it into medical practice.

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Conflict of Interests

The authors declare no conflict of interest.

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ПРОТИЗАПАЛЬНІ ЕФЕКТИ ПРОПОКСАЗЕПАМУ НА РІЗНИХ МОДЕЛЯХ ЗАПАЛЕННЯ

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ФІЗИКО-ХІМІЧНИЙ ІНСТИТУТ ІМ. О.В. БОГАТСЬКОГО НАЦІОНАЛЬНОЇ АКАДЕМІЇ НАУК УКРАЇНИ, ОДЕСА, УКРАЇНА

Вступ. *Пропоксазепам, 7-бром-5(2-хлорфеніл)-3-пропокси-1Н-бензо[е][1,4]діазепін-2(3Н)-он розглядається, як перспективний анальгетик і антиконвульсант, і станом на сьогодні проходить доклінічні випробування.*

Мета. *Вивчити протизапальні властивості та аналгетичну активність пропоксазепаму.*

Методи. *Протизапальна активність була визначена на моделі карагінан-індукованого набряку лапи щурів, викликаної формаліном відповіді лизання у мишей та брадикінінової моделі болю у щурів.*

Результати. *Вперше було продемонстровано, що застосування пропоксазепаму викликало значний протизапальний ефект у різних тестах in vivo викликаного хімічними речовинами запалення, а саме карагінану, брадикініну та формаліну.*

Висновки. *Пропоксазепам значно зменшує інтенсивність гострого та підгострого запалення та проявляє протизапальну активність, співставну з референтними препаратами.*

КЛЮЧОВІ СЛОВА: **пропоксазепам; диклофенак натрію; протизапальна дія; карагінан; формалін.**

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IMPACT OF NITRIC OXIDE SYNTHESIS MODULATORS ON THE CYTOKINES PROFILE IN EXPERIMENTAL ANTIPHOSPHOLIPID SYNDROME

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Background. *Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of specific antibodies.*

Objective. *The aim of the study was to investigate the effect of combined use of L-arginine and aminoguanidine on cytokine profile (IL-1β, IL-6, TNF-α, IL-4, IL-10) in experimental APS.*

Methods. *The study was performed on BALB/c female mice. L-arginine (25 mg/kg) and aminoguanidine (10 mg/kg) were used for correction. Serum cytokines concentrations were assessed using an ELISA test.*

Results. *It was found that in APS the concentration of proinflammatory cytokines IL-1β, IL-6 and TNF-α increases in 3.2, 2.3 and 4.5 times respectively, compare to the control. At the same time a decrease of the IL-4 and IL-10 in 1.9 and 2.2 times was evidenced.*

Aminoguanidine, a selective iNOS inhibitor, caused a significant decrease of TNF-α by 57% ($p<0.001$), but there were no changes in IL-1β, IL-6, IL-4 and IL-10 compare to the APS-group. L-arginine combined with aminoguanidine caused a significant decrease in the concentration of IL-1β by 30% ($p<0.01$), IL-6 – by 16% ($p<0.05$), TNF-α – by 59% ($p<0.001$) compare to the control. At the same time, the concentration of IL-4 increased by 35% ($p <0.01$), IL-10 – by 25% ($p<0.005$).

Conclusions. *Combined use of the precursor of the NO synthesis L-arginine and aminoguanidine, a selective iNOS inhibitor, leads to a decrease in the concentrations of IL-1β, IL-6, TNF-α and an increase of IL-4 and IL-10 compare to the group of the BALB/c mice with APS and the group of animals administered with aminoguanidine.*

KEY WORDS: **antiphospholipid syndrome; cytokines; nitric oxide; L-arginine; aminoguanidine.**

Introduction

Antiphospholipid antibody syndrome (APS) is an autoimmune condition characterized by the presence of antiphospholipid antibodies (aPL) [1], encompassing primary APS, secondary APS, seronegative APS (SNAPS) and catastrophic APS (CAPS) [2]. Secondary APS can be found in association with other autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroid disease, Crohn's disease, Sjogren syndrome, systemic sclerosis, lymphoma or leukemia, malignancies of the ovary and cervix, drug induced as with oral contraceptive pills or in infectious disease such as HIV or syphilis [1]. In CAPS a systemic inflammatory response, systemic endothelial dysfunction and DIC develop. These processes are the pathogenetic basis for development of multiple organ failure [3, 4]. SNAPS is negative for lupus anticoagulant and antiphospholipid antibodies [2].

The diagnostic APS criteria are anticardiolipin (aCL), antiβ2-glycoprotein-I (aβ2GPI) and lupus anticoagulant (LA) [1, 5]. APS can be classified only in the presence of thrombotic (non-inflammatory arterial, venous or small vessel thrombosis) obstetric complications (death of one or more morphologically normal fetus at or beyond the 10th week of gestation; one or more premature birth of normal fetus before the 34th week due to eclampsia, pre-eclampsia or placental insufficiency), or increased aPL level [2]. The mechanisms of thrombosis in APS have not been fully studied yet [6].

aβ2GPI antibodies are central in pathogenic APS mechanisms and, although the full pathogenesis of APS is not clear yet, the binding of these aPL antibodies to the antigens on the cell surface of platelets, monocytes, endothelial cells and trophoblasts triggers intracellular signaling with subsequent activation and alteration

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of diverse cell functions. Cellular activation starts after the binding of the complex aβ2GPI antibody/β2GPI [5, 7]. β2GPI is the most important antigenic target [2]. Platelets activation and the subsequent release of thromboxane favor their aggregation. Thrombosis at the fine vasculature of the target organ is thought to be more dependent from antibodies against the anticoagulant AnV. Endothelial cells and monocytes activation determine a pro-aggregation status due to up-regulated expression of adhesion molecules, such as E-selectin, and release of tissue factor (TF) and proinflammatory cytokines [5]. Many patients with aPL antibodies remain asymptomatic [2].

An important factor in APS immunopathogenesis is dysregulation of cytokine balance with increased synthesis of proinflammatory cytokines [8, 9].

Cytokines are the most versatile system of regulation. Cytokines, being synthesized at the inflammation site, affect virtually all cells involved in the inflammation development, as well as granulocytes, macrophages, fibroblasts, endothelial cells, epithelium cells, T and B lymphocytes [10].The inflammatory processes are controlled by the proinflammatory (IL-1, IL-2, IL-6, IL-8, IL-12, TNF-α, IFN) and anti-inflammatory (IL-4, IL-10, TGF) cytokines [11]. Therefore, the study of pathobiochemical mechanisms of APS development, particularly establishment of the role of the cytokine system in development of this pathology, and search for effective methods of its treatment is an urgent and social issue [1, 6, 11, 12].

One of the links that are involved in the mechanisms of APS development is the nitric oxide (NO) system. In obstetric APS, the synthesis and bioavailability of nitric oxide (NO), which is involved in the regulation of vascular tone and blood coagulation properties, are impaired in the endothelium [4]. According to Cella M [13], a decrease in NO levels causes abortion and premature birth. On the other hand, NO overproduction mediated with inducible NO synthase (iNOS) increases uterine contractions and the risk of miscarriage [13]. Contradictions of the existing information on the involvement of the NO system in APS development as well as on the efficacy of NO precursors in reducing the manifestations of this pathology necessitates further study of the role of this system in APS.

The **objective** of research is to investigate the effect of combined use of L-arginine and aminoguanidine on cytokine profile (concentration of IL-1β, IL-6, TNF-α, IL-4, IL-10) in experimental antiphospholipid syndrome.

Methods

Female BALB/c mice, which were kept on a standard vivarium diet, were used in the research. The experiments were carried out following the principles of bioethics according to the "General Ethical Principles of Animal Experiments", approved by the First National Congress on Bioethics (Kyiv, 2001) and in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and EU Directive 2010/10/63 EU for animal experiments.

APS was modeled using cardiolipin (Sigma, USA), which was injected intramuscularly four times (30 μg per 1 injection, the injection interval was 14 days) [14]. To enhance the effectiveness of the immune response, cardiolipin was emulsified in 75 μl of complete Freund's adjuvant (first injection); subsequent injections were performed with incomplete Freund's adjuvant. APS was developing for 2 weeks after the last cardiolipin injection.

The experimental animals were divided into 4 groups: the 1st – the intact; the 2nd – the BALB/c mice with APS; the 3rd – the animals with APS administered with aminoguanidine, the 4th – the animals with APS administered with L-arginine in combination with aminoguanidine. L-arginine (Sigma, USA, 25 mg/kg) and aminoguanidine (Khimlaboratorreaktiv, Ukraine, 10 mg/kg) were administered intraperitoneally once a day for 10 days after APS development. The animals of the control group were managed with the same volumes of the solvent intraperitoneally. In 10 days after confirmation of APS the animals were taken out of the experiment by thiopental sodium anesthesia (intraperitoneal administration of 1% solution at a dose of 50 mg/kg of animal body weight).

The concentration of cytokines IL-1β, IL-6, TNF-α, IL-10, IL-4 in the serum of BALB/c mice was determined by enzyme immunoassay using standard kits adapted for mice of Express Biotech International, USA (Mouse IL-1β ELISA Assay, Mouse IL-6 ELISA Assay, Mouse TNF-α ELISA Assay, Mouse IL-10 ELISA Assay, Mouse IL-4 ELISA Assay). The concentration of cytokines was expressed in pg/ml.

Statistical processing of digital data was performed by means of Excel software (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA) using non-parametric methods of estimation

for the attained data. The arithmetic mean (M), its variance and standard error of the mean (m) were assessed for all parameters. The significant difference between the independent quantitative values was determined using the Mann-Whitney test. The changes were statistically significant at $p \leq 0.05$.

Results

According to the attained results, an increase of the concentration of IL-1β in 3.2 times ($p<0.001$) was proved in the BALB/c mice with APS compare to the control (Fig. 1).

An increase in the concentration of IL-6 in 2.3 times ($p<0.001$) in the serum of the animals with APS was evidenced compare to the intact animals (Fig. 2).

TNF-α concentration increased in 4.5 times ($p<0.001$) in the serum of the BALB/c mice with APS compare to the control (Fig. 3).

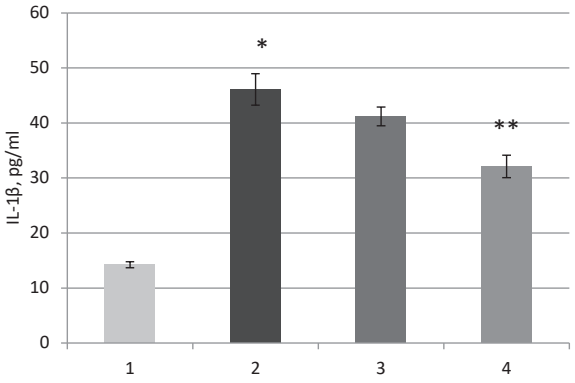

Fig. 1. IL-1β content in the blood serum of the BALB/c mice with APS in the case of administration of L-arginine and aminoguanidine (M±m, n=10).
Notes: Herein, and Figures 2-5.
Conventional name of animal groups: 1 – Control; 2 – Antiphospholipid syndrome (APS); 3 – APS + aminoguanidine; 4 – APS+L-arginine+aminoguanidine.
** – p<0.05 compare to the control group;*
*** – p<0.05 compare to the group of animals with APS.*

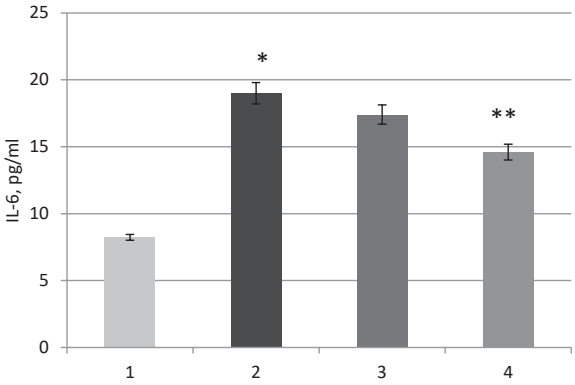

Fig. 2. IL-6 content in the blood serum of the BALB/c mice with APS in the case of administration of L-arginine and aminoguanidine (M±m, n=10).

At the same time, the anti-inflammatory cytokine IL-4 concentration reduced in 1.9 times ($p<0.001$) and IL-10 – in 2.2 times ($p<0.001$) compare to the control (Fig. 4-5).

The administration of aminoguanidine, a selective iNOS inhibitor, did not cause significant changes concentrations of IL-1β and IL-6 in the serum of the BALB/c mice with APS compare to the control (Fig. 1-2). In the case of aminogua-

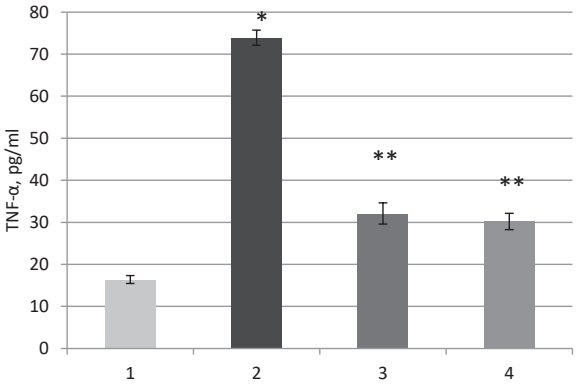

Fig. 3. TNF-α content in the blood serum of the BALB/c mice with APS in the case of administration of L-arginine and aminoguanidine (M±m, n=10).

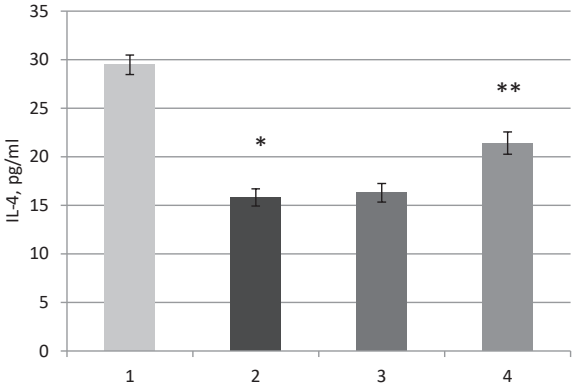

Fig. 4. IL-4 content in the blood serum of the BALB/c mice with APS in the case of administration of L-arginine and aminoguanidine (M±m, n=10).

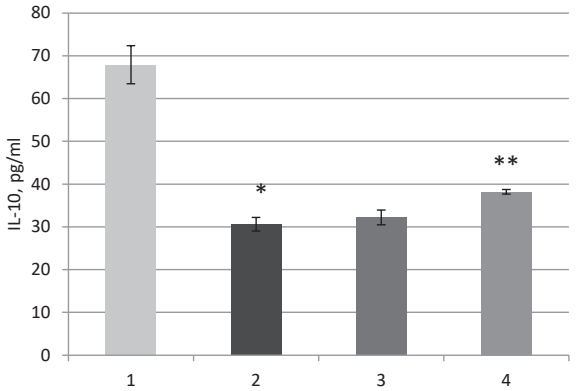

Fig. 5. IL-10 content in the blood serum of the BALB/c mice with APS in combined administration of L-arginine and aminoguanidine (M±m, n=10).

nidine use, a significant decrease in the concentration of TNF-α by 57% (p<0.001) was evidenced compare to the intact animals (Fig. 3). It was found out that, under the influence of aminoguanidine, the concentrations of IL-4 and IL-10 did not change significantly compare to the control group of animals (Fig. 4-5).

In the case of administration of the predecessor of the synthesis of NO L-arginine in combination with aminoguanidine, a significant decrease in the concentration of IL-1β by 30% (p<0.01), IL-6 by 16% (p<0.05), TNF-a by 59% (p<0.001) was established compare to the control (Fig. 1-3). At the same time, the concentration of anti-inflammatory cytokines IL-4 increased by 35% (p <0.01) and IL-10 – by 25% (p<0.005) compare to the control animals (Fig. 4-5).

The results of the study proved that a significant decrease in the concentration of IL-1β by 22% (p<0.05), IL-6 by 23% (p<0.005) was evidenced in the case of combined administration of L-arginine and aminoguanidine compare to the indicators of the 3rd group of animals, which were administered with aminoguanidine (Fig. 1-2). An increase of the anti-inflammatory cytokine IL-4 concentration by 32% (p<0.05) and IL-10 by 19% (p<0.05) was proved compare to the 3rd group of the BALB/c mice administered with aminoguanidine (Fig. 4-5).

Discussion

Besides the pathogenic role of the aPL, pro-inflammatory cytokines and chemokines are significant in the pathogenesis of APS [12]. IL-1, TNF-a and endotoxins induce tissue factor (TF) expression in endothelial cells, monocytes, macrophages promoting blood clotting [10, 15]. The inhibitors of IL-1 production are IL-4, IL-10, IL-12, TNF-α [16]. IL-6 is involved in regulation of T and B cell interactions, macrophage, endotheliocytes activity. IL-6 induces production of acute-phase proteins, stimulates hematopoiesis and platelet formation [16].

The attained results on increased concentrations of proinflammatory cytokines (IL-1β, IL-6, TNF-α) in the serum of the experimental animals with APS conform with the literature [6, 17, 18]. According to N.V. Seredavkina [6], an increased concentration of IL-6 and TNF-α in the patients with APS compare to the control group was established. It is not clear whether aPL affect endothelial cells directly or through TNF-α. Regardless of the mechanism, the prothrombotic condition, typical of APS, is associated with both significantly increased aPL levels as well as high TNF-α concentration [11]. According to J. Swadzba et al. [15] TNF-α is one of the main proinflammatory cytokines in APS; its level is increased and reflects pathological processes in endothelial cells. According to the literature, aPL and TNF-a can activate the endothelium and induce prothrombotic phenotype of endothelial cells, leading to increased thrombin production. Activation of endothelial cells causes upregulation of TF, which has been suggested to be a major potential mechanism of APS-related thrombosis. Once endothelial cells are activated, TF regulation can be more enhanced by a synergizing effect of TNF-a and factor Xa, thus expression of adhesion molecules (ICAM-1, VCAM-1, E and P selectins) and formation of endothelial microparticles take place [15].

According to A. Farzaneh-Far et al. [17], who investigated the levels of CRP IL 6, ISAM-1, pTNF α-P2, pTNF α-P2 in the patients with SLE, only increased pTNF α-P1 and pTNF α-P2 were associated with aPL positivity. According to NV Seredavkin a negative correlation between CRP and IgG β2GP1 levels was established [6]. R.R. Forastiero et al. [18] established that IL 6 levels were greater in the patients with APS and aPA carriers than in the control group. TNF concentration was the same in the patients with APS and aPA carriers but higher than in the control group. In the patients with positive aPA, a direct correlation between IL 6 and TNF α was proved [18]. Under the experimental conditions it has been established that TNF-a may manifest antiplatelet and antithrombotic activity [15]

According to the literature, IL-1β activates the synthesis of IL-6, S100B, α1-antihymotrypsin, inducible nitric oxide synthase (iNOS) causing increased NO synthesis [19, 20]. The iNOS is crucial in the primary proinflammatory response in macrophages [21]. AG is a nucleophilic hydrazine compound, structurally similar to L-arginine in that these compounds contain two chemically equivalent guanidino nitrogen groups and to L-arginine analogues that competitively inhibit NO synthase. AG completely prevents inflammatory stimuli induced formation of NO, and it is a potent inhibitor of the cytokine-inducible isoform NOS [22].

The results of our studies proved that introduction of aminoguanidine, a selective iNOS inhibitor, did not cause significant changes in the concentration of proinflammatory cytokines (IL-1β and IL-6) and anti-inflammatory cytokines (IL-4 and IL-10) in the serum of the BALB/c mice

with APS compare to the control group of animals. At the same time, in the case of aminoguanidine administration a significant decrease in TNF-α concentration was proved compare to the intact animals. According to EI Ferreira et al. [23] aminoguanidine decreases TNFα levels, oxidative stress indicators, and NO metabolites.

It is established that increased concentrations of TNF-α are associated with pregnancy miscarriage in APS [6, 8], endotheliocytes activation, and chemokine amplification that leads to subendothelial leukocyte accumulation, endothelial dysfunction, microcirculation disturbances [16].

Early endothelial dysfunction was observed in APS [24]. Patients with APS displaying thrombosis exhibited low plasma levels of nitrites and nitrates, which are the stable metabolites of NO breakdown. aPL can act as antagonists of endothelial nitric oxide synthase (eNOS) through β2GPI, and this interaction may impair NO synthesis. In particular, attenuation of eNOS activation by aPL was mediated by reduced phosphorylation of eNOS serine. This inhibition of eNOS phosphorylation was shown to be dependent upon protein phosphatase 2A, β2GPI, and apolipoprotein E receptor 2. aPL inhibition of eNOS activity contributes to thrombus formation, increased leukocyte adhesion, and alterations in vascular tone [4].

It is established that violation of the bioavailability of NO may be one of the causes of endothelial dysfunction. This may be associated with both the lack of substrate for NO L-arginine synthesis as well as formation of superoxide anion which rapidly binds and inactivates NO [24].

NO synthesis is not dependent on L-arginine concentration in physiological states. In pathological conditions, the availability of L-arginine may determine production of NO. It is proved that L-arginine is necessary for adequate translation of iNOS. When iNOS is being activated, superoxide anion is produced, which forms a highly reactive peroxynitrite, which in turn produces nitrosylation of amino acid residues sensitive to it, especially tyrosine, that leads to conformational changes in the structure of protein molecules. With administration of L-arginine the functional characteristics of T-cells enhance, production of antibodies increases as well. NO-dependent effect of L-arginine on the immune system may be hormone-mediated [25].

The next objective of our study was to investigate the effect of combined use of L-arginine and aminoguanidine on cytokine profile in APS.

In the case of the use of the precursor of NO L-arginine synthesis in combination with aminoguanidine, a selective iNOS inhibitor, a significant decrease in the concentration of proinflammatory cytokines (IL-1β, IL-6, TNF-a) and an increase in the concentration of anti-inflammatory cytokines (IL-4, IL-10) was established compare to the control group of animals. The attained results are consistent with the literature [25, 26]. These effects can be explained by the fact that glutamine formed from L-arginine is a conditionally essential amino acid and reduces the level of TNF-α soluble receptors [26]. According to VM Sheibak et al. [25] administration of L-arginine decreases the level of IL-6.

According to P. Soltesz et al. [12], besides the conventional Th1 pathway, Th2 cytokines are crucial in the mechanisms of APS development, i.e. IL-4 and IL-10. Various immunocompetent cells regulate the proinflammatory cascade that leads to cytokine imbalance and activated circulating lymphocytic pool in APS. This proinflammatory process leads to endothelial dysfunction, development of arterial and venous thrombosis [12]. As a result of the research, a decrease in anti-inflammatory cytokines (IL-4, IL-10) in APS was established; the results are consistent with the literature [11, 12]. According to P. Soltesz et al. [12], the markers of endothelial dysfunction positively correlate with IL-4 levels in APS. It allows suggesting that by activation of the humoral and cellular immune responses, IL-4 is crucial in development of endothelial dysfunction, atherosclerosis, arterial and venous thrombosis. IL-4 stimulates B and T cell proliferation as well as differentiation of CD4 + T cells into Th2 cells [12].

According to A. Menachem et al. [11] cytosolic and secreted IL-10 and IFN-γ levels in eAPS mice were lower at 6 and 15 weeks and higher at 24 weeks after immunization compared to adjuvant mice. IL-10 is significant in autoimmune diseases. As a result of other studies, IL-10 level was decreased in the serum of the patients with APS [12].

IL-10 inhibits secretion of IL-4, IL-5 and IFN-γ, growth factors and chemokines, and therefore acts as a key counter-regulator of autoimmune processes [12]. One of the functions of IL-10 is inhibition of the synthesis of proinflammatory cytokines: IL-1, IL-6, IL-12 and TNFα via a STAT3-dependent mechanism [27] and enhancement of IL-1 receptor antagonist

expression [19]. Decreased level of IL-10 in the serum in cases of APS compare to the control confirms the fact that IL10-mediated processes are impaired in APS that is why it leads to vascular damage [12].

Low IL-10 levels enable TNF-α unregulated production, resulting in procoagulant state. Decreased IL-10 levels can be associated with lymphocyte activation, which leads to the continuation of the autoimmune response. During the B-cell activation, IL-10 delivers signals that promote the apoptosis of B cells [11].

Conclusions

Thus, that in the serum of BALB/c mice with APS, an increase in the concentrations of proinflammatory cytokines (IL-1β, IL-6, TNF-a) and a decrease in the concentrations of anti-inflammatory cytokines (IL-4 and IL-10) was established compare to the control parameters. With the introduction of aminoguanidine, a selective iNOS inhibitor, a decrease in the concentration of TNF-α was proved compare to that of the animals with APS. In the case of the use of the precursor of NO synthesis L-arginine in combination with aminoguanidine, a significant decrease in concentrations of IL-1β, IL-6, TNF-α and an increase of IL-4 and IL-10 was evidenced compare to the group of BALB/c mice with APS and the group of animals administered with aminoguanidine.

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Conflict of Interests

The authors declare no conflict of interest.

Authors Contributions

Yaremchuk O.Z. – writing – original draft, conceptualization, project administration, methodology, investigation, formal analysis, *Posokhova K.A.* – supervision, conceptualization, writing – review & editing, *Kuzmak I.P.* – data curation, *Kulitska M.I.* – investigation, *Shevchuk O.O.* – investigation, writing – review & editing, *Volska A.S.* – investigation, *Lykhatskyi P.H.* – data curation.

ВПЛИВ МОДУЛЯТОРІВ СИНТЕЗУ ОКСИДУ АЗОТУ НА ПОКАЗНИКИ ЦИТОКІНОВОГО ПРОФІЛЮ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ АНТИФОСФОЛІПІДНОМУ СИНДРОМІ

О.З. Яремчук, К.А. Посохова, І.П. Кузьмак, М.І. Куліцька, О. О. Шевчук, А.С. Вольська, П.Г. Лихацький

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Антифосфоліпідний синдром (АФС) – це автоімунне захворювання, яке характеризується наявність антифосфоліпідних антитіл, артеріальними та венозними тромбозами, тромбоцитопенією, невиношування вагітності.*

Мета дослідження. *Дослідити вплив комбінованого застосування L-аргініну та аміногуанідину на показники цитокінового профілю (концентрацію IL-1β, IL-6, TNF-α, IL-4, IL-10) при експериментальному антифосфоліпідному синдромі.*

Методи дослідження. *Дослідження виконано на мишах-самках лінії BALB/c, в яких моделювали АФС. Для корекції використовували L-аргінін (25 мг/кг) та аміногуанідин (10 мг/кг). Визначення концентрації цитокінів IL-1β, IL-6, TNF-α, IL-10, IL-4 у сироватці крові мишей BALB/c проводили методом імуноферментного аналізу з використанням стандартних наборів реактивів.*

Результати й обговорення. *Отримані результати свідчать, що у сироватці крові мишей BALB/c за умов АФС відбувається зростання концентрації прозапальних цитокінів IL-1β у 3,2 раза, IL-6 у у 2,3 раза, TNF-α в 4,5 разів, відносно контролю. Спостерігалось зниження концентрації протизапальних цитокінів IL-4 в 1.9 раза та IL-10 в 2,2 раза у групі тварин з АФС, порівняно із показниками контролю.*

На фоні застосування селективного інгібітора iNOS аміногуанідину встановлено достовірне зниження концентрації TNF-α на 57 % ($p<0.001$), проте концентрація IL-1β, IL-6 IL-4 та IL-10 достовірно не змінювалася у сироватці крові мишей BALB/c з АФС, порівняно з показниками тварин з АФС. На фоні застосування попередника синтезу NO L-аргініну в комбінації з аміногуанідином встановлено достовірне зниження концентрації IL-1β на 30 % ($p<0.01$), IL-6 на 16 % ($p<0.05$), TNF-α на 59 % ($p<0.001$), відносно контролю. Водночас зростала концентрація протизапальних цитокінів IL-4 на 35 % ($p<0.01$) та IL-10 на 25% ($p<0.005$), порівняно з показниками групи мишей BALB/c з АФС.

Висновки. *Встановлено, що комбіноване застосування попередника синтезу NO L-аргініну та селективного інгібітора iNOS аміногуанідину призводить до зниження концентрації IL-1β, IL-6, TNF-α та зростання IL-4 та IL-10, порівняно з показниками групи мишей BALB/c з АФС та групи тварин, яким вводили аміногуанідин.*

КЛЮЧОВІ СЛОВА: **антифосфоліпідний синдром; цитокіни; оксид азоту; L-аргінін; аміногуанідин**

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ROLE OF ENDOGENIC INTOXICATION IN MUSCLE INJURY IN EXPERIMENT

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Background. *Endogenous intoxication is a multicomponent complex process due to the endogenous biological products or dysfunction of systemic natural detoxification.*

Objective. *The aim of the research was to study the dynamics of indices of endogenous intoxication in rats with traumatic muscle damage in the experiment.*

Methods. *The experiment was performed on 45 non-linear white rats, which were modeled with traumatic muscle damage. The level of endogenous intoxication was assessed by the content of medium plasma molecules (MMM), leukocyte and erythrocytic index of intoxication (LII and EII). The research was conducted on the 1st, 3rd, 7th, 14th days after the injury.*

Results. *It was found that traumatic muscle damage causes endotoxemia. Manifestations of endogenous intoxication are: the increase of MMM1 in 2.3 times, MMM2 in 2.8 times compare to the intact animals. The level of this indicator slightly decreased in 7 days. Simultaneously with an increase in the MCT level in the post-traumatic period, the total toxic effect on the erythrocyte membrane also increased, which was manifested by a significant increase in EII in all terms of observation.*

Conclusions. *Traumatic damage of the muscles is accompanied by the growth of molecules of average mass in upto 7 days of observation, which significantly differ from the indicators of the intact group. The results of our research prove that traumatic muscle damage causes endotoxicosis development evidenced by accumulation of endotoxins in the animals' body that is proved by significant changes in endogenous intoxication indices: i.e. erythrocytic and leukocytic indexes of intoxication and content of medium mass molecules.*

KEY WORDS: **endogenous intoxication; traumatic muscle damage; middle mass molecule.**

Introduction

The increase in injuries all over the world is still one of the topical socio-economic issues of today [1,3,4]. Every year in Ukraine, about 10% of the population gets an injury of varying degrees of severity. Mortality from accidents and injuries in Ukraine increases by an average of 1% annually [2]. According to the World Health Organization, the traumatic damages are the third among the causes of mortality, and among the population under 40 years – the first. Despite the fact that the patients with polytrauma make up 8-10% of all inpatient cases, they account for 68% of fatal cases. According to the data of the European Commission's newsletter in 2019, about five people are seriously injured with consequences in life-threatening road accidents. Serious injuries are often more expensive for society through long-term rehab and medical needs [1]. At the same time, mortality from accidents and injuries is constantly increasing: an average of 1% annually [2], which is evidence of the ineffectiveness of medical care provided to these patients. Endogenous intoxication is a complex multicomponent process due to the pathological biological activity of endogenous products or dysfunction of systemic natural detoxification [5,6]. When the body is injured, there are significant general and local changes that are considered within the traumatic disease [2]. The morphological substrate of traumatic illness is the damage of organs and tissues of various localization and character, which arise with excessive mechanical influence [2, 4].

At the moment of injury, tissue elements are destroyed or damaged, receptor fields are changed, and the integrity of blood and lymphatic vessels is violated. Releasing physiologically active substances, in particular, proteolytic enzymes and biogenic amines causes secondary damage. Oxidative stress causes damage to the body of biomacromolecules that leads to accumulation of products of oxidative modification of proteins, degradation of lipid

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components, nucleotides, pigments, and formation of a significant number of middle mass molecules (MMM) [7]. To date, there are three stages of development of the syndrome of systemic inflammatory response: the stage of local production of mediators in response to injury, which can be regarded as a protective response (healing of wounds, protection of cells from pathogenic microorganisms); a stage of ejection of a small number of mediators into the bloodstream to support homeostasis; the stage of generalization of the inflammatory reaction, in which the regulatory systems are not able to provide the homeostasis of the organism [8]. The above mediators exhibit destructive functions, primarily in the system of endothelial cells.

One of the systems of the body that is undergoing significant changes in trauma is the detoxification system. This system dysfunction leads to development of endogenous intoxication syndrome (EIS), which accompanies diseases and complications associated with increased tissue disintegration, increased catabolism, internal organs insufficiency [9]. Markers of endogenous intoxication are molecules of average mass, erythrocyte index of intoxication, which is established and fast in execution [9,10]. To date, the age-related mechanisms of the development of endogenous intoxication syndrome is still unclear, there are no perfect pathogenetic approaches to explaining and predicting the early and late effects of traumatic lesions.

Methods

The experiment was performed on 45 nonlinear white rats, males, weighing 180-200 g, which were kept on a standard vivarium diet. The rats were kept and all experiments were performed following the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes" (Strasbourg, 1986); The General Ethical Principles of Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001), the Helsinki Declaration of the World Medical Association (2000).

The animals were divided into 2 groups: 1 – the intact (12 animals), 2 – the controls with traumatic muscle damage (33 animals). The injury was modeled by a dose-fetched foot thigh, equivalent to the animals by the severity of the injury in thiopental-sodium general anesthesia (40 mg/kg). Animals on the 1st, 7th (early), 14th (intermediate), 21st (late) days of the post-traumatic period were taken from the experiment. Euthanasia of rats was performed by decapitation under thiopental anesthesia with subsequent total hemorrhaging. The level of endogenous intoxication was assessed by the content of medium-mass molecules in plasma, defining of leukocyte index of intoxication (LII) and erythrocytic indices of intoxication (EII). The content of middle mass molecules was determined in accordance with the method [7]. An acid-soluble fraction was isolated from the blood serum, which was obtained by adding 1.8 ml of a 10% solution of trichloroacetic acid to 0.2 ml of serum. The middle mass molecules content was determined at wavelengths of 254 (chain amino acids were determined) and 280 nm (aromatic amino acids were determined), then the coefficient Kc (MMM 280 nm / MMM 254 nm) was calculated.

The leukocyte index of intoxication is the ratio of blood cell populations that indirectly allows evaluating the predominance of response to pro-or anti-inflammatory cytokines. The leukocyte index of intoxication was determined according to the formula suggested by Ya. Kalf-Kalif in modification by B.A. Reis [10].

Endogenous intoxication was determined by a technique based on the idea of erythrocytes as a universal adsorbent, which allows assessing the level of endogenous intoxication by changing the sorption capacity of erythrocytes of the polar, practically not penetrating through their methylene blue membrane. The amount of absorbed color (in percentages) [7] was calculated using the formula:

$$A=100-C\times100/B,$$

where A – the amount of absorbed dye, %;

B – the optical density of the initial solution, conditional units of extinction;

C – the optical density of the dye solution after incubation with erythrocytes, conditional units of extinction.

Statistically significant differences between the control and experimental groups were estimated using Mann-Whitney's non-parametric criterion. The differences were considered significant at the probability of a zero hypothesis less than 5% ($p<0.05$).

Results

According to the results of the case study, the MMM1 and MMM2 indices that reflected the content of chain and aromatic amino acids in the medium-sized peptides respectively as well as their decomposition products increased by the 1st day after trauma (Table 1). Thus, the

content of MMM1 in the blood of the affected rats increased in 2.3 times compare to the intact animals. In the same experiment period, the content of MMM2 in rat blood increased accordingly in 2.8 times in relation to the intact animals. The most significant changes in the content of both MMM fractions were observed in 24 hours after the injury. In 7 days after the injury, there was a slight tendency to decrease in the MMM content. Thus, under the influence of the injury, the concentration of blood concentration in both MMM1 and MMM2 was noted. Moreover, the MMM method was more significant for the blank MMM2, indicating the severity of the aromatic amino acids of the average molecule. Since MMMs were the markers of endotoxicosis, the significant changes in their content evidenced the peak of the development and generalization of the syndrome.

The features of MMM are their clearly expressed high biological activity. The accumulation of MMM is not only a marker of endotoxicosis; in the future, they increase the course of the pathological process, acquiring the role of secondary toxins, affecting the livelihoods of all systems and organs. The level of MMM is considered the main biochemical marker, which reflects the level of pathological protein metabolism. It does not only accompany acute and chronic pathology, but is an important factor in their pathogenesis, determines the course and consequences of the disease. According to the results of the research (Table 2), simultaneously with the increase in the MMM level in the post-traumatic period, the total toxic effect on the erythrocyte membrane also increased, which was manifested by a significant increase in endogenous intoxication in all terms of observation.

It is established that the degree of destruction of membranes of erythrocytes during the experiment was the highest. Changes are obviously repeated by the fact that when injuries are caused to the body, energy metabolism and transport of substances in erythrocytes are disturbed, the permeability of their membrane progressively increases.

Based on the analysis of blood cell parameters, the leukocyte index of intoxication was calculated at the beginning of the experiment and in its different terms. As a result of the study, an ambiguous response of the leukocyte relating the pathological processes development was established in a day: the leukocyte index of intoxication increased in 2.7 times (Table 3).

Leukocyte index of intoxication by Ya. Calf-Caliph in B.A. Reis modification increased and amounted to (0.75±0.01)% on the 7th day of the post-traumatic period and (0.25±0.01)% in the control.

Discussion

According to the case results, MMM1 and MMM2 increased in up to 1 day after trauma. The content of MMM1 in the blood of the affected rats increased in 2.3 times (0.575±0.031) compare to the intact animals (0.250±0.014), and the MMM2 content in the rat blood increased accordingly in 2.8 times (0.484±0.011)

Table 1. Dynamics of the content of medium mass molecules (MMM) in the serum of the rats with traumatic muscle damage (M ± m)

Indicator	Animal groups				
	Intact (n=12)	Terms of observation			
		1st day (n=30)	7th day (n=27)	14th day (n=26)	21st day (n=25)
MMM1, (conditional units)	0.250±0.014	0.575±0.031*	0.514±0.026*	0.414±0.026*	0.341±0.026*
MMM2, (conditional units	0.173±0.001	0.484±0.011*	0.450±0.021*	0.350±0.021*	0.270±0.021*
Coefficient MMM2/ MMM1	0.692	0.841*	0.875*	0.845*	0.791

*Note: * – p<0,05 – the probable differences compared with the intact animals.*

Table 2. Dynamics of erythrocytic index of intoxication (EII) (%) in the rat blood (M ± m)

Indicator	Animal group				
	Intact (n=12)	Terms of observation			
		24 hours (n=30)	7th day (n=27)	14th day (n=26)	21st day (n=25)
(EII) %	33.1±3.8	62.1±1.1*	73.5±8.0*	47.72±2.0*	40.20±1.0*

*Note: * – p<0.05 – the probable differences compared with the animals of the intact.*

Table 3. Dynamics of leukocyte index of intoxication (%) in the blood of rats (M+m)

Indicator	Animal groups				
	Intact (n=12)	Terms of observation			
		24 hours (n=30)	7th day (n=27)	14th day (n=26)	21st day (n=25)
Leukocyte index of intoxication (%)	0.25±0.02	0.67±0.01	0.75±0.01	0.41±0.01	0.27±0.01

*Note: * – p<0.05 – the probable differences compared with the animals of the intact.*

relative to the intact animals (0.173±0.001). The most significant changes in the increase of MMM were observed in 24 hours after the injury. In 7 days after the beginning of the experiment, there was a slight tendency to decrease in the content of MMM (MMM1 0.514±0.026, MMM2 0.450±0.021). Intoxication syndrome is caused by trauma and is accompanied by increased tissue breakdown, increased catabolic processes, due to the accumulation of excessive amounts of biologically active substances, deformed protein metabolites and other toxic substances of endogenous origin. the results of the research proved that simultaneously with the increase in the MMM level in the post-traumatic period, the total toxic effect on the erythrocyte membrane also increased that was manifested by a significant increase in endogenous intoxication in all terms of observation.

As a result of the research, an ambiguous reaction of the leukocyte response to the development of pathological processes was established in a day: the leukocyte index of intoxication increased in 2.7 times 0.25±0.02, and the increase on the 7th day of the post-traumatic period was significant 0.75±0.01 compared with the control 0.25±0.01. The increase of this indicator may evidence the activation of inflammatory reactions in the area of traumatic muscle damage and the activation of reparative processes.

Conclusions

Traumatic damage of the muscles is accompanied by the growth of molecules of average mass up to 7 days of observation, which sig_ nificantly differ from the indicators of the intact group. The results of our research indicate that traumatic muscle damage causes endotoxicosis development, which is evidenced by accumulation of endotoxins in the body of animals that is proved by significant changes in endogenous intoxication indices: erythrocyte and leukocyte indexes of intoxication and content of medium mass molecules.

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Conflict of Interests

The authors declare no conflict of interest.

Authors Contributions

Dzhyvak V.H. – Investigation, Formal analysis, writing – original draft; *Khlibovska O.I.* – investigation, formal analysis, validation, writing – original draft; *Klishch I.M.* – conceptualization, supervision, writing – review and editing.

МАРКЕРИ ЕНДОГЕННОЇ ІНТОКСИКАЦІЇ ПРИ ТРАВМАТИЧНОМУ УРАЖЕННІ М'ЯЗІВ В ЕКСПЕРИМЕНТІ

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ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Ендогенна інтоксикація – це складний багатокомпонентний процес, зумовлений патологічною біологічною активністю ендогенних продуктів або дисфункцією систем природної детоксикації, що викликає як загальні, так і локальні зміни.*

Мета дослідження. *Вивчити динаміку показників ендогенної інтоксикації у щурів з травматичним ушкодженням м'язів.*

Методи дослідження. *Експеримент проводили на 45 нелінійних білих щурах. Нанесення травми відбувалося в умовах тіопентало-натрієвого знечулення (40 мг/кг) шляхом дозованого удару по стегну. Рівень ендогенної інтоксикації оцінювали за вмістом молекул середньої маси в плазмі, визначення лейкоцитарного індексу інтоксикації (ЛІІ) і еритроцитарного індексу інтоксикації (ЕІІ). Дослідження проводили на 1, 3, 7, 14 день після травми.*

Результати. *Було виявлено, що в результаті травматичного ураження м'язів розвивається ендотоксикоз, свідченням чого є накопичення ендотоксинів в організмі тварин. Проявами ендогенної інтоксикації є зростання МСМ1 у 2,3 рази, МСМ2 у 2,8 рази відносно інтактних тварин. Рівень даного показника незначно зменшувався на 7 добу. Одночасно із збільшенням у посттравматичному періоді рівня МСМ, зростав і сумарний токсичний вплив на мембрани еритроцитів, який проявлявся в достовірному підвищенні ЕІІ у всі терміни спостереження.*

Висновки. *Травматичне ушкодження м'язів супроводжується зростанням молекул середньої маси до 7 доби спостереження які достовірно відрізняються від контрольних показників. Результати експериментального дослідження свідчать, що при травматичному ушкодженні м'язів розвивається ендотоксикоз, свідченням чого є нагромадження ендотоксинів в організмі тварин, на що вказують виражені зміни показників ендогенної інтоксикації – еритроцитарного та лейкоцитарного індексів інтоксикації та вмісту молекул середньої маси.*

КЛЮЧОВІ СЛОВА: **ендогенна інтоксикація; травматичне ушкодження мязів; молекули середньої маси.**

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POSITIVE EFFECT OF ENTEROSORPTION IN DOXORUBICIN-INDUCED CARDIOHEMODYNAMICS ALTERATION

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Background. *Anthracycline antibiotics are one of the most effective anti-cancer drugs, but their cardiotoxicity what limits its therapeutic use.*

Objective. *To analyze the efficiency of enterosorption in doxorubicin-induced cardiohemodynamics violation.*

Methods. *Subchronic doxorubicin toxicity was modeled by injecting the anthracycline antibiotic intraperitoneally at a dose of 5 mg/kg once a week for 4 weeks, in total 20 mg/kg. Male Wistar rats were randomly distributed into 3 groups: control; DOX-group and DOX + enterosorbent C2 rats (γ=0.18 g/cm^3, BET area 2162 m^2/g). Cardiohemodynamics was studied by the Millar Instruments, heart morphometry – by Avtandilov's method.*

Results. *Mortality rate in DOX-group was 25%. Ejection fraction and Stroke work indices were lower compared to the control group, preload adjusted maximal power decreased by 57.6%, minimum volume and end-systolic volume increased by 76,2 and 67.5% respectively. End-systolic stiffness of left ventricle (E_{max}) as well as arterial elastance (E_a) and end-systolic pressure had tended to decrease. Indices of left ventricle (LV) volume at systole increased: V@dPdt$_{max}$ – by 73.3%, V@dPdt$_{min}$ – by 81.9%. End-diastolic volume increased by 54.6%. As for the dPdt$_{min}$, and Tau constant we observed the slight tendency to its decline. Endocardial surface of LV increased by 42.7%, Planimetric Index – by 40.4% compared to the control group of rats.*

In DOX+C2 group mortality rate was 18.75%. We observed the strong tendency to normalization of the main indices compared to the DOX group and shrinking of the LV. We want to underline the positive trends especially in Ejection Fraction (from 39.62±10.50% to 46.23±11.46%) and Stroke Work (from 6406.50±3345.83 to 10363.14±7329.55 mmHg×uL) as important indicators of the effectiveness of cardiac pump function.

Conclusions. *Enterosorption demonstrated positive impact on the doxorubicin-induced violated cardiohemodynamics and decreased the mortality rate. It is a ground for further investigations.*

KEY WORDS: **doxorubicin-induced subchronic toxicity; heart damage; enterosorption; cardiohemodynamics parameters.**

Introduction

Anthracycline antibiotics are widely used to treat many types of malignancies because of their high efficacy. But, also, they are cardiotoxic, what limits their therapeutic use and cumulative dose [1,2]. Doxorubicin (Adriamycin, a derivative of rubomycin – 14-hydrorubomycin) is a part of chemotherapy schemes for treatment of breast and prostate cancer, solid tumors in children, sarcomas, and others. Multiple mechanisms of heart damage by anthracyclines are recognized. Oxidative stress and generation of reactive oxygen species (ROS) by "anthracycline-iron" complex; cardiac muscle's accumulation of highly reactive alcoholic metabolite doxorubicinol (DOXol); cytokines disturbances; as a consequence of endogenic intoxication and bacterial translocation because of mucositis – are only a few possible ways of cardiomyocytes injury [3–7].

Till today there are no definite and 100% efficient methods for prevention and treatment of anthracyclines-induced cardiotoxicity. Iron-chelating agent dexrazoxane was implemented into protocols based on its capability to prevent free radical release [8,9]. But there are some facts that this agent could decrease the efficacy of anti-cancer chemotherapy [10]. That is why the search of effective means to ameliorate the cardiotoxicity of anthracyclines, which do not

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attenuate the anti-tumor activity of drugs, remains actual. Sorption Detoxification is a well-known method for cleaning of body fluids from toxic endogenous or exogenous compounds. The most widely used types of this method are the purification of blood or its components (hemosorption), oral administration of sorption materials (enterosorption), and application-sorption therapy of wounds and burns [11]. Our previous studies with enterosorbents Carboline and carbon granular oral adsorbent C2 demonstrated promising results to alleviate the side effects caused by cytostatic agents melphalan and cisplatin (bone marrow suppression, gastrointestinal toxicity, testes damage, etc.) [12–15]. Enterosorbent C2, which has optimized and shifted to mesopores porous structure, in combination with an officinal biosimilar of granulocyte colony-stimulating factor (filgrastim) ameliorated hematologic toxicity and oxidative stress indexes much better than each of these preparations alone [16].

The objective of this study is the assessment of the capability of carbon granular oral adsorbent C2 to diminish the doxorubicin-induced heart damage.

Methods

Materials

Doxorubicin hydrochloride (Doxorubicin Teva 10 mg/5ml, concentrate for solution for infusion, TEVA Pharmachemie, the Netherlands) was used for experiments. Carbon oral adsorbent C2 was specially designed at the Department of Means and Methods of Sorption Therapy of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR). Parameters of enterosorbent C2 are next: bulk density γ=0.18 g/cm^3, granules with a diameter of 0.15–0.25 mm, the porous structure of C2 is well developed and shifted toward mesopores, which surface is 565 m^2/g. BET (Brunauer-Emmett-Teller) surface area is 2162 m^2/g.

Animal studies

All experiments were carried out with male Wistar rats, 180-220 g of primary weight, which were reared at TNMU animal facility (Ternopil, Ukraine). All procedures were done according to the local bioethical committee guidelines which conform to the rules and requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and EU Directive on the Use of Animals for Research Directive 2010/63/EU. A common light-dark cycle was maintained for rats and fed on common rodent chow diet with tap water *ad libitum*, according to the guidelines for animal care.

A well-documented regimen was used for the induction of heart damage by doxorubicin [17]. Animals were randomly assigned to 3 groups: 1) control group (n=7); 2) rats treated with DOX (DOX-group) (n=16); 3) rats treated with both DOX and carbon enterosorbent C2 (DOX+C2) (n=16).

Subchronic doxorubicin toxicity was modeled by injecting the anthracycline antibiotic intraperitoneally at a dose of 5 mg/kg once a week for 4 weeks, in total 20 mg/kg [17]. The animals serving as control received the same volume of saline intraperitoneally once a week for a total of 4 weeks. Newly designed carbon oral adsorbent C2 was given into the stomach via a custom rigid tube once a day at a dose of 5 ml per kg (or 1 ml for each 200 g of rat body weight; or 900 mg of the dry mass of the enterosorbent). We started enteral sorption therapy the next day after the first injection of Doxorubicin. The sorbent was given as a suspension in an appropriate volume of distilled water. The rats of the control group received an equal volume of distill water. On the days of doxorubicin injection and one day before it, the enterosorbent was not given to avoid any pharmacokinetics disruption.

Cardiohemodynamics measurements

For the direct cardiac function evaluation, we used Millar pressure-volume (P-V) system (MPVS-300, Millar Instruments, Houston, TX, USA). On the 29th day of the experiment counting from the first injection of doxorubicin, under urethane general anesthesia (1.5 g/kg) the right carotid artery was exposed and ligated distally, the artery was clamped and incised, and a 0.5 cm long 90 PE tube was inserted as a catheter guide. A 2-Fr Mikro-Tip catheter (SPR-838, Millar Instruments, Houston, TX, USA) was advanced through the guide into the LV under pressure control; a ligature was then tightened around the catheter to avoid blood loss [18]. After stabilization for 5 min, signals were continuously sampled at a sampling rate of 1000 samples/sec by the MPVS-300 system, recorded, and displayed on a personal computer by the PowerLab System and ChartTM v.5.4.2 software (ADInstruments, Millar Instruments) for 15-20 min.

The relation of pressure and volume of the left ventricle was performed by software PVAN 3.6 (AD Instruments, Millar Instruments) with the conversion of relative volume units (RVU) into absolute one (equation slope 20,25×RVU –

intercept 29,05). The Millar P-V System simultaneously and continuously measures left ventricle (LV) pressure (P) and volume (V) from the beating heart, producing characteristic PV loops readings of which a variety of cardiovascular parameters, such as heart rate (HR), cardiac output (CO), stroke volume (SV), ejection fraction (EF), stroke work (SW), dP/dt_{max}, and dP/dt_{min} are derived. End-systolic pressure (ESP), end-systolic volume (ESV), end-diastolic pressure (EDP), end-diastolic volume (EDV), stroke volume (SV), stroke work (SW), maximum dP/dt ($dPdt_{max}$), minimum dP/dt ($dPdt_{min}$), tau, maximum dV/dt ($dVdt_{max}$), minimum dV/dt ($dVdt_{min}$), maximum pressure (Pmax), minimum pressure (Pmin), maximum volume (Vmax), and minimum volume (Vmin) were also analyzed.

Morphometrics of the heart

To estimate chronic changes of the shape and size, the hearts of the rats were used for measuring and evaluating of the planimetric index. For indirect planimetry of the endocardial surface of the rats' hearts, ventricles were taken accordingly to Avtandilov G.G. method[19] in Esypova I.K. et al. modification [20]. We measured the endocardial surfaces of the left (E_{LV}) and right ventriculi's wall area (E_{RV}). Planimetric index (PI) was calculated as:

$$PI=E_{LV}\div E_{RV}.$$

where E_{LV} is the endocardial surfaces of the left ventricle wall area and E_{RV} is the endocardial surfaces of the right ventricle wall area.

Statistical analysis

The normality of data distribution was tested using Kolmogorov-Smirnov test, homogeneity of variance – Levene's test. Mann-Whitney test and One-way ANOVA was applied to test the differences between the groups. Statistical analysis was performed using Microsoft Excel XP (USA) and Statistica 10.0 (StatSoft Inc., USA). Differences were considered significant if the probability of Type I error was less than 0.05. $P<0.05$ was considered significant.

Results

In DOX-group the number of prematurely deceased rats was four, in DOX+C2 group – three rats died before the end of the experiment. All abovementioned animals died after the 4th injection of doxorubicin during the last week of the experiment. Among survived rats we observed typical clinical signs of heart failure: rats showed clear signs of dyspnea, from mild to severe ascites, different stages of hydrothorax and liver enlargement; their common activities were reduced compared to the rats of the control group. Those sings had less intensity compared to the untreated group of animals.

The pump function of the heart was analyzed by next parameters: ejection fraction, stroke volume and stroke work and cardiac output, as well as maximal power and preload adjusted maximal power (PaMP) (table 1). These parameters are load-dependent and consequently represent poor contractility indices. Increased cardiac output, high heart rate as well as stroke volume are the typical signs of cardiac dysfunction and followed systemic hemodynamic changes and our results supposed it. It was a strong tendency for increasing of the all abovementioned indices, but enteral sorption therapy partly disrupts it. Ejection fraction in DOX-group rats was lower, but in the group DOX+C2, we see the tendency to its normalization. The same tendency was for the stroke work: from 6406.50±3345.83 in rats, which received injections of doxorubicin, it increased to 10363.14±7329.55 mmHg×uL for rats which got oral adsorbent concomitantly. While the index in rats of the control group was 7036.43±5036.46 mmHg×uL.

Minimum volume increased by 76,2%, end-systolic volume – by 67.5%. For both indices, we observed the tendency for decreasing by oral adsorbent therapy, but they did not come close to the numbers of control group rats.

In the rats of DOX-group the index of maximal power did not change significantly, but the strong tendency to its decreasing we saw, while preload adjusted maximal power (PaMP) was lower by 57.6% compared to the control group. Enteral sorption therapy promoted the tendency to normalization of the indices. End-systolic stiffness of left ventricle (E_{max}) had a strong tendency to decreasing from 4.55±2.93 to 2.74±2.02 (what means that left ventricle was dilated and lost end-systolic elastance), while carbon oral adsorbent C2 increased this index to 5.30±0.44.

Specific parameters as the volume at the point of maximal speed of pressure change ($V@dPdt_{max}$) and volume at the point of maximal speed of pressure decline ($V@dPdt_{min}$) are used for assessment of LV volume at systole. So, $V@dPdt_{max}$ increased by 73.3%, while $V@dPdt_{min}$ – by 81.9%.

During diastole, the myocardium stops shortening and generating force and relaxes. Diastolic function was analyzed by changes of end-diastolic pressure and volume, the peak rate of pressure decline ($dPdt_{min}$) – isovolumic relaxation, constant Tau by Weiss method (τ_w).

Table 1. Cardio-hemodynamics indices in rats, which received doxorubicin and enteral sorption therapy with oral carbon adsorbent C2.

Index	Control group	DOX group	DOX + C2 group
HR (min^{-1}) heart rate	321.0±43.89	356.64±48.82	378.38±33.86
Maximum Volume (uL)	146.79±13.08	232.76±76.58	236.68±102.07
Minimum Volume (uL)	78.27±15.17	137.93±46.96*	126.05±35.68
End-systolic Volume (uL)	84.87±17.73	142.16±47.56*	130.76±36.68
End-diastolic Volume (uL)	142.56±11.36	220.44±70.94*	223.75±85.04
Maximum Pressure (mmHg)	116.24±21.53	102.0±22.51	111.85±14.35
Minimum Pressure (mmHg)	4.97±3.89	4.15±3.39	2.10±1.65
End-systolic Pressure (mmHg)	109.57±25.12	96.22±24.07	104.58±16.72
End-diastolic Pressure (mmHg)	9.10±4.28	6.72±4.54	5.99±3.77
Stroke Volume (uL)	68.52±21.34	94.835±39.90	120.88±78.45
Ejection Fraction (%)	46.15±11.83	39.62±10.50	46.23±11.46
Cardiac Output (uL/min)	22222.85±8424.99	33103.55±13814.04	44721.45±28511.89
Stroke Work (mmHg×uL)	7036.43±5036.46	6406.50±3345.83	10363.14±7329.55
Arterial Elastance (Ea), (mmHg/uL)	2.25±0.71	1.06±0.49	1.11±0.56
dPdt max (mmHg/sec)	11758.0±5232.28	9897.43±3142.76	12769.50±2861.17
dPdt min (mmHg/sec)	-7312.86±2477.79	-7062.50±1742.62	-8928.12±3274.57
dVdt max (uL/sec)	2811.14±1048.07	4783.57±1703.76	4489.87±2985.50
dVdt min (uL/sec)	-3345.57±1283.04	-3893.5±1345.52	-4751.13±2491.48
P@dVdt max (mmHg)	38.27±32.11	40.16±35.79	34.62±36.27
P@dPdt min (mmHg)	88.67±50.41	64.69±13.78	74.00±21.68
V@dPdt max (uL)	124.90±25.62	216.46±76.45*	228.02±102.85*
V@dPdt min (uL)	79.91±15.49	145.33±52.64*	129.76±36.71
Tau(W) (msec)	12.69±5.87	11.41±5.01	9.25±1.96
Tau(G) (msec)	18.34±11.18	13.80±5.68	11.90±3.39
Maximal Power (mWatts)	43.89±34.61	38.94±18.71	57.61±32.30
Preload adjusted maximal power, PaMP (mWatts/μL^2)	20.81±15.88	8.82±4.78*	12.38±5.77
E_{max}	4.55± 2.93	2.74±2.02	5.30±0.44

Notes. The data are expressed as means (M) ± standard deviation (SD); – p<0,05 comparing to control group; $dPdt_{max}$ – peak rate of pressure rise; $dPdt_{min}$ – peak rate of pressure decline; $dVdt_{max}$ – peak rate of volume rise; $dVdt_{min}$ – peak rate of volume decline; $P@dVdt_{max}$ – Pressure at dV/dt max; $P@dPdt_{min}$ – Pressure at dV/dt_{min}; $V@dPdt_{max}$ – Volume at dP/dt_{max}; $V@dP/dt_{min}$ – Volume at dP/dt_{min}; Tau (G) – relaxation time constant calculated by Glantz method (regression of dP/dt versus pressure); Tau (W) – relaxation time constant calculated by Weiss method (regression of log(pressure)); E_{max} – end-systolic elastance.*

End-diastolic volume increased by 54.6% in the group of rats, which received doxorubicin compared to the control rats. As for the $dPdt_{min,}$ we observed a slight tendency to its decline, as well as for the Tau constant.

After 4 injections of Doxorubicin on 29th day of the experiment, the endocardial surface of the left ventricular wall area increased by 42.7% (p<0.001) compared to control group of rats (table 2). At the same time, there were no changes in the right ventricular wall area. In rats which received enterosorption together with doxorubicin, the endocardial surface of the left ventricular wall area index decreased

Table 2. The influence of enterosorption on morphometric indexes of the heart ventricles in subchronic doxorubicin toxicity in rats.

Index	Control group	DOX-group	DOX+C2 group
The endocardial surface of left ventricular wall area, mm^2	118.0±4.45	168.4±7.63*	132.6±3.06*, **
The endocardial surface of right ventricular wall area, mm^2	132.2±6.27	134.8±6.54	137.8±4.88
Planimetric index (PI)	0.894±0.010	1.25±0.032*	0.965±0.022**

*Notes: The data are expressed as means (M) ± standard error (SE). p<0.05 compared to * – control group; ** – DOX-group.*

by 21.3% compared to the DOX group, but it was still larger than in rats of the control group.

Doxorubicin injections increased the Planimetric index (PI) the by 40.4% compared to the control group (from 0.89±0.01 to 1.25±0.03), while in DOX+C2 group it decreased by 22.8% (0.96±0,02, $p<0.001$).

Discussion

Our study deals with the effect of enterosorption on doxorubicin-associated cardiac toxicity. Doxorubicin's use in patients is limited by its cardiac toxicity. Today a new subspecialty appeared – cardio-oncology, which focuses on prevention, detection, monitoring, and treat_ment of cardiovascular pathology during anticancer chemotherapy [21]. It is a marker of the high importance of this problem because long term survival of childhood cancers is more than 70% for now [6] and continued to increase [22]. Strong links between cancer and heart disease are recognized, that is why a clinical need for optimized cardio-oncology patient management is growing. Among anti-cancer agents, the most capable drugs to cause the dilative cardiomyopathy are anthracyclines and cyclophosphamide [16,23]. Dose-dependent irreversible heart damage occurs in 1.7% of patients mostly via oxidative stress activation and by inhibition of transcriptions of genes, which are responsible for the synthesis of the contractile proteins [3,16]. Up to 3% of heart transplantations were done for patients because of doxorubicin therapy [6]. It is known that the prognosis of patients who develop doxorubicin-induced congestive heart failure is poor: approximately ~50% mortality in 1 year [3]. Monoclonal antibody trastuzumab and low molecular tyrosine kinase inhibitors as sunitinib and sorafenib may cause heart damage too [23]. They modulate mitochondrial integrity, deplete ATP and lead to contractile dysfunction. But in this case, the contractile function of the left ventricle improves after drugs discontinuation [23].

We used typical widespread modeling to induce congestive heart failure in rats: four injections of DOX at the dose of 5 mg/kg for cumulative dose 20 mg/kg and got the cardiohemodynamic disruption [17]. So, this model could be used for assessment of the capability of different substances and drugs to impact the heart systolic and diastolic function. One of the experimental morphometric methods to measure and estimate the type and deepness of heart injury is weighing and weight measurement of different parts of the organ namely left and right ventricle with the septum (ventricle index, Fulton index, etc.). The planimetric method allows estimating changes of both ventricles by measuring the endocardial surfaces area [19]. And this method is validated to estimate the chronic changes of the heart morphology, while cardiohemodynamics violations measured by Millar Instruments are quite good for assessment of acute functional changes in heart work.

A wide variety of indexes that can be quantified by analyzing pressure-volume (PV) loops have been proposed to characterize the left ventricle systolic and diastolic performance. In the present study, doxorubicin-associated cardiac dysfunction was manifested by a reduction in cardiac systolic and diastolic hemodynamic function. We have shown statistically significant differences between DOX and control groups in parameters of end-systolic and end-diastolic volumes as well as volumes at the point of maximal speed of pressure change and pressure decrease. Also, we have shown a 57.6% decrease in Preload adjusted maximal power. Doxorubicin at the cumulative dose of 20 mg per kg promoted the heart dilation, which was confirmed by increased indices of the endocardial surface of the left ventricular wall area and planimetric index. Our previous study demonstrated the decreased mass of the heart in subchronic doxorubicin toxicity. So, despite only the slight tendency of ejection fraction declining, these important changes already indicate the onset of the dilated cardiomyopathy. Such results are supported by research on the male New Zealand white rabbits with doxorubicin-induced heart damage [24]. So, we may conclude, that early myocardial effects of doxorubicin-induced cardiotoxicity are presented. We may talk about early stages of dilated cardiomyopathy with still preserved ejection fraction, but with clinical signs of congestion in survived rats – non-failing dilated left ventricle in survived animals. Our results are confirmed by the study of Lodi M. et al. [25]: A significantly reduced ejection fraction was seen on day 80 only. They modeled cardiomyopathy by 6 IV injection of DOX at the dose of 1.5 mg/kg on the 8^{th}, 11^{th}, 14^{th}, 17^{th}, 20^{th} and 23^{rd} days of the experiment [25]. Also, the results of our histological examination of heart tissues presented revealed loss of myofibrils and striations, as well as cytoplasmic edema.

Our previous study demonstrated that enterosorption with C2 ameliorates the morphological sings of heart damage [26]. Also, we

observed improvements of hematological parameters, kidney's function and decrease of endogenous intoxication markers. Those data are in press.

It is important to mention that the concomitant course of enterosorption during this experiment decreased the mortality rate. In rats which received doxorubicin, it was 25% (4 rats from 16), while at the DOX+C2 group – 18.75% (3 rats from 16).

DOX+C2 rats' group has shown a statistically significant difference compared to the control group in the parameter of volume at the point of maximal speed of pressure change. $V@dPdt_{max}$ increased by 82.6%.

All 26 parameters of cardiohemodynamics were altered in rats which received doxorubicin at the total dose of 20 mg/kg. More than a half (14 parameters) among them demonstrated tendency to normalization under the influence of enteral sorption therapy. Especially we want to notice the positive tendency in indices of Preload adjusted maximal power, PaMP (from 8.82±4.78 to 12.38±5.77 mWatts/μL^2), Maximal Power (from 38.94±18.71 to 57.61±32.30 mWatts, and it was even higher than in the control group – 43.89±34.61 mWatts), Stroke Work (from 6406.50±3345.83 to 10363.14±7329.55 mmHg×uL, while the control group index was 7036.43± 5036.46 mmHg×uL) and Ejection Fraction from 39.62±10.50% to 46.23±11.46%, when in the control group it was 46.15±11.83%).

Conclusions

Doxorubicin at the total dose of 20 mg/kg caused pronounced violation of cardiohemodynamics. Systolic indices as Ejection fraction, stroke work, end-systolic elastance (E_{max}), end-systolic pressure – all these indices demonstrated a tendency to decline, preload adjusted maxi_mal power (PaMP) was lower by 57.6% compared to the control group. It is a marker of weaker pump function and poor contractility of the heart. Morphometry showed dilation of the left ventricle and increased planimetric index. At the same time, the diastolic indices were disrupted too. End-diastolic volume significantly increased by 54.6%, the index of peak rate of pressure had a tendency for declining, as well as Tau(w). The indices of volume at the point of maximal speed of pressure change ($V@dPdt_{max}$) and volume at the point of maximal speed of pressure decline ($V@dPdt_{min}$) significantly increased in rats, which received doxorubicin. It confirms the diastolic dysfunction presence.

Enteral sorption therapy mostly normalized and improved violated indices and decreased the rate mortality of rats. We observed shrinking of the endocardial surface of the left ventricular wall area by 21.3% and decreasing of Planimetric Index. Those results demonstrate that enterosorption could prevent remodeling of the heart chambers. Our cardiohemodynamics investigations included more than 20 parameters and though mostly they are not statistically significant we want to underline the positive trends in DOX+C2 rats comparing to DOX-group, especially in Ejection Fraction and Stroke Work parameters as they are the important indicators of the effectiveness of cardiac pump function. Such results could be explained by the fact that measurement of hemodynamics was done one week later after the last 4th injection of doxorubicin, and we observed the consequences of mechanisms of adaptation in survived rats.

Our research demonstrated promising results of the efficiency of carbon granular oral adsorbent C2 to ameliorate the doxorubicin-associated cardiohemodynamics changes and are the ground for further future investigation of different combinations of enterosorption and cardio-tropic drugs.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Shevchuk O.O. – investigation, conceptualization, resourses, writing – original draft; *Portnichenko G.V.* – formal analysis, visualization, investigation, writing – original draft, data curation*, Lapikova-Bryginska T.Y.* – investigation, data curation*, Goncharov S.V.* – investigation, data curation; *Nikolaev V.G.* – conceptualization, project administration, writing (review and editing), supervision*; Dosenko V.E.* – project administration, writing (review and editing), supervision.

ПОЗИТИВНИЙ ВПЛИВ ЕНТЕРОСОРБЦІЇ НА ПОРУШЕННЯ КАРДІОГЕМОДИНАМІКИ, СПРИЧИНЕНІ ДОКСОРУБІЦИНОМ

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1 – ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНІ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

2 – ІНСТИТУТ ФІЗІОЛОГІЇ ІМЕНІ О.О, БОГОМОЛЬЦЯ НАН УКРАЇНИ, КИЇВ, УКРАЇНА

3 – ІНСТИТУТ ЕКСПЕРИМЕНТАЛЬНОЇ ПАТОЛОГІЇ, ОНКОЛОГІЇ І РАДІОБІОЛОГІЇ ІМЕНІ Р.Є. КАВЕЦЬКОГО НАН УКРАЇНИ, КИЇВ, УКРАЇНА

Вступ. *Кардіотоксичність протипухлинних лікарських засобів, і особливо антрациклінових антибіотиків, є одним з лімітуючих факторів ефективного лікування злоякісних новоутворів.*

Мета. *Дослідити можливості ентеросорбції для пом'якшення кардіогемодинамічних змін, викликаних доксорубіцином в експерименті.*

Методи. *Субхронічна доксорубіцинова токсичність моделювалася чотирьохкратним введенням доксорубіцину інраперитонеально в дозі 5 мг/кг один раз на тиждень протягом 4 тижнів у сумарній кумулятивній дозі 20 мг/кг. Щури були рандомізовані у 3 групи: контроль, група тварин, що отримувала доксорубіцин (DOX-група) та групу, котра окрім останнього отримувала ентеросорбент С2 (γ=0.18 г/см^3, BET – 2162 м^2/г). Параметри кардіогемодинаміки вивчалися за допомогою Millar Instruments, морфомтричні зміни серця – за методом Автанділова.*

Результати. *Летальність у DOX-групі склала 25%. Показники фракції викиду та ударної роботи серця знижувалися порівняно з показниками контрольної групи. Показник максимальної потужності, зрівноваженої на переднавантаження був достовірно нижчим на 57,6%, а мінімальний об'єм та кінцево-систолічний об'єм зросли на 76,2 та 67,5%, що свідчить про розвиток застійних явищ. Показники $V@dPdt_{max}$ зросли на 73.3%, $V@dPdt_{min}$ – на 81.9%. Кінцево-діастолічний об'єм був вищим на 54.6%. Спостерігалася тенденція до зниження $dPdt_{min}$ та Таи константи. Ендокардіальна поверхня лівого шлуночка зросла на 42,7%, а планіметричний індекс – на 40,4%.*

У групі DOX+C2 летальність склала 18,75%. Спостерігалася виражена тенденція до нормалізації усіх показників. Особливо ми хочемо підкреслити позитивний ефект застосування вуглецевого ентеросорбента С2 на показники фракції викиду (з 39.62±10.50% до 46.23±11.46%) та ударної роботи (з 6406.50±3345.83 до 10363.14±7329.55 мм.рт.ст×мкл) як важливих показників насосної функції серця.

Висновки. *В статті наведені дані, котрі демонструють здатність ентеральної сорбційної терапії зменшувати зрушення показників кардіогемодинаміки, спричинені введенням доксорубіцину. Окрім цього, ентеросорбція сприяла зменшенню показника летальності піддослідних тварин.*

КЛЮЧОВІ СЛОВА: **субхронічна доксорубіцинова токсичність; пошкодження серця; ентеросорбція; параметри кардіогемодинаміки.**

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GENDER-SPECIFIC DIFFERENCES OF CARDIAC VEGETATIVE CONTROL IN ADRENALINE-INDUCED NECROSIS AND LIGHT DEPRIVATION

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Background. *Cardiovascular disease is the main cause of morbidity predominantly in males. Stress is one of the crucial factors, especially with light desynchronosis.*

Objective *of the study was to assess gender-specific characteristics of cardiac vegetative control in myocardial necrosis in cases of light deprivation.*

Methods. *Cardiac vegetative control in adrenaline-induced myocardial necrosis (AIMN) in a setting of light deprivation (LD) was assessed in 72 mature white rats of both sexes. The animals were divided into 2 groups: G1 – the animals kept under day/night cyclic balance (12 hours/12 hours); G2 – the animals kept at LD (illumination 0.5-1 LX) for 10 days. On Day 11, AIMN caused by adrenaline (0.5 mg/kg) and heart rate variability (HRV) was assessed in 1 hour and 24 hours.*

Results. *The development of AIMN at LD in the ♂ G2 led to HRV increase that was caused by augmentation of parasympathetic and reduction of sympathetic cardiac effects. In cases of AIMN, changes of CVC in the ♀ G2 were similar to the ♀ G1. However, in 1 hour of AIMN, parasympathetic cardiac effects were more significant than in the ♀ G1. While the ♀ G2 AIMN animals experienced balanced sympathetic and parasympathetic actions, the predominance of the sympathetic component was evidenced in the ♀ G1 AIMN animals.*

Conclusions. *Light deprivation has different effects on baseline sympathetic/parasympathetic balance in males and females, i.e. increased parasympathetic control of heart rhythm in males and maintenance of sympathetic/ parasympathetic balance in females.*

KEY WORDS: **myocardial necrosis; heart rhythm variability; light deprivation; gender.**

Introduction

According to the WHO data, cardiovascular disease is the most frequent cause of death in the European population; moreover, it has frequent severe sequelae, leads to disability and reduces the quality of life. Ukraine is on the list of countries in Eastern Europe, where coronary artery disease (CAD) mortality in people of 55-60 years of age is higher than that in French subjects 20 years senior [1]. The leading causes of increased mortality include ageing population and lifestyle factors, especially smoking and suffering from overweight [2]. Males are more frequently affected by coronary artery disease than females. This difference, however, is found in the middle-aged persons. At menopause, the number of females in the population with coronary episodes and myocardial infarction increases greatly. This might be associated with deficiency of oestrogens and their diminished cardioprotective and vasoprotective activity [3].

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Important risk factors of myocardial necrosis include impaired circadian rhythms and functional disorders of the pineal gland [4, 5]. This is related to professional activity, sleep disorders and difficulty falling asleep [6, 7]. Similar to all other systems in the body, the cardiovascular system is controlled by the pineal gland. Its activity changes depending on the phase of the circadian cycle (light/darkness), as suggested by fluctuations in blood pressure and heart rhythm variability [5, 8]. However, the effects of melatonin synthesised by the pineal gland vary in males and females that is associated not only with the ability of this hormone to regulate the synthesis of sex hormones [4, 9, 10], but also to its capability to change the activity of sympathetic and parasympathetic components of the autonomic nervous system (ANS) [11, 12]. Murine experiments have demonstrated different ANS responses to melatonin in males and females [11]. A higher basal tone of the parasympathetic component in female ANS contributes to a better cardioprotective effect of melatonin when adrenaline-induced myocardial necrosis is modelled in a

setting of melatonin [13]. A similar trend was found in humans. Analysis of ANS status (as reported by a 24-hour monitoring of heart rhythm variability) suggests reduced parasympathetic tone and increased sympathetic activity as predictors of fatal arrhythmias and sudden death in subjects with myocardial infarction [14]. The benefits of heart rhythm variability analysis and its diagnostic and prognostic value are beyond doubt. The use of this method allows assessment of cardiac vegetative control in a study of pathogenetic role of light desynchronosis as a contributor to myocardial necrosis. However, the available data do not provide a full understanding of the role of ANS in adaptation of heterosexual organisms to abnormal light conditions, including the development of cardiac disease under these conditions.

The **objective** of the study was to assess gender-specific characteristics of cardiac vegetative control in adrenaline-induced myocardial necrosis in rats in cases of light deprivation.

Methods

The experiments were conducted according to ethical guidelines approved by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Order of the Ministry of Health of Ukraine No. 690, dated September 23, 2009. The tests were performed on 72 nonlinear male and female white rats (body weight: 220-270 g; age: 5-5.5 months). The rats were provided with a standard laboratory pellet diet and water and were housed in plastic cages at constant ambient temperature and humidity. The rats of the control group (group 1, 18 ♂, 18 ♀) were kept under a natural day/night cycle (light 12 hours, 500 LX/night 12 hours, 0.5-1 LX). The rats of the experimental group (group 2, 18 ♂, 18 ♀) were kept in permanent darkness (0.5-1 LX) for 10 days. Necrosis of myocardium was modelled by administration of adrenaline (intramuscular, 0.5 mg/kg of body weight) on day 11. In 1 and 24 hours after adrenaline administration, EKG was captured (2 standard leads) using a Cardiolab-CE computer-based complex (Kharkiv, Ukraine). The durations of 1000 consecutive cardiac R-R intervals accurate to 0.001 second were assessed. In order to evaluate the regulatory activity of sympathetic and parasympathetic ANS components on the heart rhythm, the following parameters were registereg: Mo (sec) = mode, i.e. the most frequently captured duration of the R-R interval; AMo (%) = the amplitude of mode, i.e. the percentage of cardiac intervals meeting the Mo value; ΔX (sec) = the difference between the largest and the smallest R-R value; HSI (abs. value) = heart strain index reflecting the degree of centralisation of heart rhythm control, this parameter is obtained from the following expression: HSI=AMo/(2·ΔX·Mo); VBI (abs. value) = vegetative balance index reflecting the ratio between the activities of the sympathetic and parasympathetic nervous system, this parameter is obtained from the following expression: VBI=AMo/ΔX; VRI (abs. value) = vegetative rhythm index reflecting the activity of the autonomic circuit of heart rhythm control, this parameter is obtained from the following expression: VRI =1/Mo·ΔX, and IARP (abs. value) = index of adequate regulation processes reflecting the balance between the activity of the sympathetic component of the autonomic nervous system and the predominant functional level of the sinus node. By matching against heart rhythm (HR), this parameter allows judgment on excessive or insufficient centralisation of heart rhythm control; this parameter is obtained from the following expression: IARP=AMo/Mo.

Statistical analysis of the results was performed using parametric method of variation statistics based on established normal distribution of data in the rows compared, with n=6 in each of the rows. The following parameters were determined by the arithmetic mean (M), standard deviation (σ) and Student's t-test (t). The difference between the arithmetic means was statistically significant at t value not less than 2.228 ($p \leq 0.05$). Microsoft Excel XP (BioStat Pro 6.7.1.0) software (US) was used for calculations.

Results

The results attained in the animals of the control group (Group 1), which were under conditions of balanced light/darkness (12 hours/12 hours), are presented in Table 1.

The development of adrenaline-induced myocardial necrosis within 1 hour after adrenaline administration was found to cause a significant increase in HR (by 12.5% in the ♂, by 13% in the ♀, $p<0.05$). This process was accompanied by a predictable reduction in Mo values (by 12% in the ♂, by 13% in the ♀). AMo values were increased in the ♂ (by 87%, $p<0.05$) and the ♀ animals by (34%, $p<0.05$). The ΔX

Table 1. Parameters of heart rhythm variability in the rats developing adrenaline-induced myocardial necrosis under the intact day/night cycle (12 hours/12 hours), M±σ

Parameter	Gender	Controls (n=6)	1 h post-adrenaline (n=6)	24 h post-adrenaline (n=6)
HR	♂	472±21#	531±17*	491±29
	♀	438±19#	496±27*	481±12*
Mo (sec)	♂	0.127±0.006#	0.113±0.002*#	0.122±0.007
	♀	0.137±0.006 #	0.121±0.007*#	0.125±0.003*
AMo (%)	♂	31.7±3.8	59.2±5.8#	43.7±7.5*
	♀	33.8±4.5	45.2±5.0*#	41.2±3.1*
ΔX (sec·10^{-2})	♂	0.65±0.22	0.35±0.08*	0.58±0.04#
	♀	0.55±0.14	0.33±0.05*	0.40±0.06*#
HSI (abs. value)	♂	21621±9813	79493±23420*	30953±6342#
	♀	23875±7223	57384±10855*	42514±9872*#
VBI (abs. value)	♂	5425±2245	17873±4936*	7527±1391#
	♀	6578±2211	13783±2142*	10598±2484*#
VRI (abs. value)	♂	1333±470	2716±935*	1418±190#
	♀	1419±442	2537±419*	2051±347*#
IARP (abs. value)	♂	0.250±0.040	0.525±0.058*#	0.358±0.061*
	♀	0.247±0.038	0.375±0.056*#	0.330±0.024*

*Note: * – a statistically significant (p≤0.05) differences relative to the controls, # – relative to the animals of the opposite sex.*

values significantly decreased in the animals of either sex (by 97% in the ♂, by 67% in the ♀, p<0.05). HSI, which is an integral parameter, increased in the animals of either sex (a 3.7-fold increase in the ♂, a 2.4-fold increase in the ♀, p<0.05). Such changes reflected the increased role of the sympathetic ANS component and the reduced influence of the parasympathetic component in heart rhythm control. These findings were confirmed by increases in VBI (a 3.3-fold increase in the ♂, a 2.1-fold increase in the ♀, p<0.05), VRI (a 2.0-fold increase in the ♂, a 1.8-fold increase in the ♀, p<0.05) and IARP (a 2.1-fold increase in the ♂, a 1.5-fold increase in the ♀, p<0.05).

In 24 hours after adrenaline administration (i.e. the peak of focal necrosis), the type of ANS response to development of the abnormal process changed somewhat. In the ♂ animals, the findings of HR, Mo, ΔX, VBI and VRI recovered to the control levels, AMo decreased (by 35%, p<0.05), while IARP were above control values (by 43%, p<0.05). In the ♀ rats, HR was higher (by 10%, p<0.05) and Mo was lower (by 10%, p<0.05). The following parameters were higher than the respective control values: AMo (by 38%, p<0.05), HSI (by 78%, p<0.05), VBI (by 61%, p<0.05), VRI (by 45%, p<0.05) and IARP (by 34%, p<0.05).

Gender-specific analysis showed Mo in the Group 1 lower in the ♂ than in the ♀ (by 8%, p<0.05). In 1 hour of necrotic process development, Mo was lower than in the ♀ (7%, p<0.05), AMo was higher (by 31%, p<0.05) and IARP was also higher (by 40%, p<0.05). In 24 hours after adrenaline administration, the ΔX in the ♂ was higher than in the ♀ (by 45%, p<0.05) and HSI, VBI and VRI were lower (by 37%, 41% and 45%, p<0.05, respectively), reflecting gender-specific differences of cardiac adjustment mechanisms in cases of ANS-mediated damage.

Analysis of parameters in the animals of the experimental group (Group 2) that were kept in permanent darkness for 10 days (light deprivation) showed that in 1 hour of myocardial necrosis development, ♂ animals had lower HR (by 9%, p<0.05), higher Mo (by 9%, p<0.05) and lower ΔX (by 23%, p<0.05) (Table 2). As for other parameters, no significant changes were evidenced. When the ♀ animals were exposed to identical conditions, HR increased (by 6%, p<0.05), while Mo and ΔX decreased (by 6% and 29%, respectively, p<0.05). Under these conditions, the increase in HSI, VBI and VBI were quite predictable (by 65%, 55% and 40%, respectively, p<0.05). IARP values did not change.

In 24 hours after adrenaline administration, the ♂ HR was lower than in the controls (by 16%, p<0.05), Mo was higher (by 16%, p<0.05) and IARP was lower (by 34%, p<0.05). In the ♀ at this phase of myocardial necrosis, HR was higher (by 6%, p<0.05), Mo was lower (by 6%,

Table 2. Parameters of heart rhythm variability in the rats with developing adrenaline-induced myocardial necrosis in cases of light deprivation, (M±σ)

Parameter	Gender	Controls (n=6)	1 hours post-adrenaline (n=6)	24 hours post-adrenaline (n=6)
HR	♂	512±14^#	469±21*^	443±29*^
	♀	435±11#	463±11*^	461±22*
Mo (sec)	♂	0.117±0.003^#	0.128±0.006*^	0.136±0.009*^
	♀	0.138±0.003#	0.130±0.003*^	0.130±0.006*
AMo (%)	♂	38.3±7.8^	34.3±3.7^	33.0±2.7^
	♀	32.7±4.4	38.3±5.2	40.5±11.6
ΔX (sec·10^{-2})	♂	0.70±0.13	0.57±0.08*^#	0.75±0.08^#
	♀	0.58±0.04	0.45±0.08*^#	0.50±0.14#
HSI (abs. value)	♂	24730±9622	24464±6557^#	16398±2345^#
	♀	20535±3552	33835±7738*^#	34836±17417*#
VBI (abs. value)	♂	5672±2123	6223±1446^#	4458±846^#
	♀	5642±939	8732±1891*^#	9118±4656*#
VRI (abs. value)	♂	1257±234	1405±244^	996±118^#
	♀	1256±90	1762±299*^	1657±517#
IARP (abs. value)	♂	0.326±0.060#	0.268±0.039^#	0.243±0.010*^
	♀	0.238±0.036#	0.296±0.043#	0.309±0.078

*Note: * – statistically significant (p≤0.05) differences relative to the controls, ^ – relative to the findings in the group 1 animals during the same observation period, # – relative to the animals of the opposite sex.*

p<0.05), HSI and VBI were higher (by 70%, 62%, p<0.05), and AMo, ΔX, VRI and IARP did not differ from the respective baseline values in this group.

Gender-specific analysis showed that HR and IARP in the control group was higher in the ♂ than in the ♀ (by 18%, 37%, p<0.05); the Mo values were lower (by 18%, p<0.05). There were no differences between the animals in terms of other parameters. In 1 hour of myocardial necrosis, ΔX in the ♂ was higher than in the ♀ (by 27%, p<0.05); HSI, VBI and IARP were lower (by 38%, 40% and 10%, p<0.05). In 24 hours of myocardial necrosis, ΔX ♂ was significantly higher (by 50%, p<0.05), and HSI, VBI and VRI were lower (in 2.1 times, 2.0 times and 1.7-times, respectively, p<0.05).

In terms of ANS responses, effects of light deprivation in development of myocardial necrosis have shown that staying in darkness for 10 days had different influences on vegetative control of heart rhythm in male and female animals. In ♂, HR increased by 8.5% (p<0.05), Mo decreased by 85% (p<0.05). Other parameters remained unchanged and were not statistically different from those in animals of Group 1. In the ♀ cohort, light deprivation did not cause any changes in investigational parameters.

Under conditions of a necrotic process (1 and 24 hours after administration of adrenaline), HR ♂ was lower than the respective values in Group 1 (13% and 11%, respectively, p<0.05); in the meantime, Mo was higher (13% and 11%, respectively, p<0.05), AMo was lower (73% and 32%, respectively, p<0.05) and ΔX was higher (63% and 29%, respectively p<0.05). All integral parameters were significantly lower than comparative ones; in part, this was true of HSI (in 3.3 times and 1.9 times, p<0.05), VBI (in 2.9 times and 1.7 times, p<0.05), VRI (in 1.9times and 1.4times, p<0.05) and IARP (in 2.0 times and 1.5 times, p<0.05). The differences between Group 1 and Group 2 in the females in 1 hour after adrenaline administration were for HR (by 7% lower, p<0.05), Mo (by 7% higher, p<0.05) and ΔX (36% higher, p<0.05); the values of HSI, VBI and VRI were lower (in1.7 times, 1.6 times and 1.4 times, respectively, p<0.05). In 24 hours after adrenaline administration, no significant differences were evidenced regarding the parameters.

Discussion

Analysis of heart rhythm variability is performed by a non-invasive method of functional diagnostics, which is used not only in a clinical setting, but also in experimental studies. It allows assessing of cardioregulatory ANS activity, determining the balance between activities of sympathetic and parasympathetic components as they affect the heart rhythm, making a conclusion about the predominant

component, and assessing the stress upon regulatory systems as part of adjustment to adverse influences [15]. Analysis of histograms reflecting the distribution of RR intervals (variational pulsography) was used in the study. Assessment of the results involved use of a cybernetic double-circuit model for heart rhythm regulation as suggested by R.M. Bayevsky: the central circuit (cerebral cortex, higher autonomic centres and the cardiovascular centre) and the autonomic circuit (sinus node, lungs and the respiratory centre) [16]. The patterns of changes in experimental findings with time allowed drawing a conclusion that significant difference in ANS effects on the heart rhythm occurred between male and female animals. In males, development of adrenaline-induced myocardial necrosis under normal day/night balance was accompanied by an increased impact of sympathetic ANS component, as evidenced by increased levels of HSI, VBI, VRI and IARP. A more significant increase in test parameters in 1 hour of the experiment was predictable due to a presence of hypercatecholaminemia caused by administration of adrenaline. The effects of adrenaline were not limited to the sinus node, but also extended to myocardial contractility. This was also true regarding its metabolite, adrenochrome [17]. The amount of adrenochrome increased significantly under our experiment as a result of catabolism and an active part of myocardial damage through stimulation of free radical processes [18]. Under the circumstances, ANS response to hypercatecholaminemia-induced oxidative stress was also a matter of discussion. This was confirmed by ipsidirectional but less significant changes in ANS responses in the females. In this model, the severity of myocardial damage was substantially lower in the females [19]. In this case, the increase in AMo reflected strengthening of the central circuit of heart rhythm regulation, and the decrease in ΔX reflected the reduced involvement of the autonomic circuit, i.e. the role of the vagus nerve. This data is consistent with the literature on using a model with adrenaline-induced myocardial necrosis. It was established that not only hypercatecholaminemia, but also sex hormones were crucial in the capacity of ANS to develop other adaptive effects [20, 21]. In 24 hours after adrenaline administration, the primary effects of adrenaline diminished according to the pattern described. In the males, all of the parameters were normal again, which was proved by a recovery of the baseline balance between ANS components. In the females, all pulsographic parameters demonstrated retention of increased sympathetic activity, which was the principal difference between the males and females.

The males of Group 2 responded to a 10-day stay in darkness with increased HR and with an accordingly reduced Mo. This reflected the state of stress and the increased involvement of humoral adaptive mechanisms, which were implemented by the adrenal glands [22]. The maintenance of regulatory balance under such conditions was attained at the expense of reduced activity of the central regulatory circuit, as confirmed by a reduction in AMo. Therefore, adjustment of the males in Group 2 to permanent darkness caused a moderate activation of sympathetic cardiac effects. In such a situation, myocardial necrosis developed under activation of the parasympathetic component and predominance of the latter in heart rhythm control. This was confirmed by higher (compare to the Group 1 animals) ΔX values and by lower values of HSI, VRI, VBI and IARP. Lower AMo values have demonstrated that the predominance of parasympathetic ANS component was also facilitated by the reduced activity of sympathetic ANS component.

A 10-day stay of the Group 2 females under conditions of permanent darkness did not cause any functional changes in the ANS, as confirmed by the absence of significant differences in all parameters in the Group 1 and Group 2 females. In 1 hour after administration of adrenaline, HR increased and Mo decreased in the females of Group 2, which reflected an increase in adrenergic effects of ANS in the heart, with the effects implemented through the humoral channel (mainly the adrenal glands). The reduction in ΔX suggested a decrease in vagal cardiac effects. As a natural result, HSI, VBI and VRI values increased. The constancy of IARP reflected a preserved balance between the central and the autonomic circuits of heart rhythm control. In 24 hours after adrenaline administration, HR was still increased, and Mo was lower than in the controls of this group. To a greater degree than in the controls, the HSI and VBI values demonstrated a predominance of sympathetic ANS effects in heart rhythm control, but the stability of IARP suggested a preserved balance between the activities of the central and the autonomic control circuits. It is important that under developing myocardial necrosis, the changes of parameters with time were similar to those

in the Group 1, although less significant. In particular, Mo was higher in the Group 2 females compare to those of Group 1, which suggested a less active involvement of adrenal glands under the simulated conditions [22]. In this case, there was no involvement of the central control circuit, as confirmed by the absence of significant AMo changes. This demonstrated a lack of response of cerebral cortex to hyperadrenalinemia. Despite the increases in HSI, VBI and VRI values in 1 hour of myocardial necrosis (which reflected a phase of hyperadrenalinemia), these values were significantly lower than in the Group 1 females. To a degree greater than in the Group 1 animals, the ΔX value reflected a more active involvement of parasympathetic mechanisms in cardiac adjustment to damage, which maintained a stable IARP and preserved a baseline balance between central and autonomic control circuits, unlike the Group 1 females, where the control balance shifted towards predominance of sympathetic ANS activity.

The development of necrotic process in the myocardium of the Group 2 males was accompanied by activation of cholinergic mechanisms of cardiac control, which was more significant than in the females. This proved that the males and females employed different mechanisms of adjustment to adverse effects of adrenaline under light deprivation. These differences may be associated with different levels of melatonin synthesis by the pineal gland under conditions of permanent darkness [9, 23]. The females synthesises more melatonin at night-time [10] and their melatonin synthesis during sleep peaked sooner than in the males [24]. Considering that all animals in the experiment were under identical conditions, higher melatonin levels in the females could be asserted. However, a clearer understanding of the significance of endogenous melatonin within a framework of adaptive responses with ANS involvement in modelling of adrenaline-induced myocardial necrosis might yield the results attained in modelling of cardiac disease under permanent lighting, which was the prospect of the research. In summary, it should be noted that the attained data have proved that the males and females have different ANS responses to developing adrenaline-induced myocardial necrosis under light deprivation that suggests the necessity to specify the gender of animals in the conclusions and the incorrectness of extrapolating the established patterns to animals of other sex.

Conclusions

A 10-day light deprivation activates the heart rhythm impact of the sympathetic component of ANS in the male rats and does not alter the baseline balance of sympathetic and parasympathetic components of ANS in the females. The development of adrenaline-induced myocardial necrosis under 10-day light deprivation is characterised by a higher (compared today/night cycle) involvement of the parasympathetic ANS component in heart rhythm control in the males and by development of bradycardia. In the females, light deprivation facilitates the maintenance of sympathetic/parasympathetic balance in cases of adrenaline-induced myocardial necrosis. This differs from the females under the day-night cycle, where an increase in the sympathetic component of the ANS was evidenced in cases of developing myocardial necrosis.

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Conflict of Interests

The authors declare no conflict of interest.

Authors Contributions

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СТАТЕВА ВІДМІННІСТЬ ВЕГЕТАТИВНОЇ РЕГУЛЯЦІЇ СЕРЦЯ ЩУРІВ ПРИ АДРЕНАЛІН-ІНДУКОВАНОМУ НЕКРОЗІ НА ТЛІ СВІТЛОВОЇ ДЕПРИВАЦІЇ

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ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Серцево-судинні захворювання є головною причиною захворюваності у світі. У когорті хворих на ішемічну хворобу серця переважають чоловіки. Серед факторів ризику головним залишається стрес, в тому ж числі на ґрунті світлового десинхронозу.*

Мета дослідження. *Вивчити гендерні особливості вегетативної регуляції серця при адреналін-індукованому некрозі міокарда в щурів на тлі світлової деривації.*

Методи дослідження. *У 72 білих статевозрілих самців і самиць щурів досліджували варіабельність серцевого ритму (ВСР) при адреналін-індукованому некрозі міокарда (АНМ) на тлі світлової депривації (СД). Тварин поділили на 2 групи: Г1 – тварини були в умовах циклу день/ніч (12 год/12 год); та Г2 – щури перебували 10 днів в умовах світлової депривації (СД). На 11-й день моделювали АНМ (адреналін в/м, 0.5 мг/кг) і ВСР вивчали через 1 та 24 год.*

Результати. *Розвиток АНМ у ♂ Г1 і ♀ Г1 викликав зменшення варіабельності серцевого ритму (ВСР), посилення симпатичних впливів на серце, що було суттєвішим в ♂. СД викликала посилення симпатичних впливів на серце в ♂ Г2. Розвиток АНМ в ♂ Г2 викликав суттєве збільшення ВСР та значне зменшення ЧСС. Це було результатом посилення парасимпатичних впливів на серце та зменшення симпатичних. У ♀ Г2 в умовах АНМ динаміка показників ВСР була аналогічною до такої в ♀ Г1. Проте, на 1 год АНМ активність парасимпатичних впливів на серце була більшою, ніж в ♀ Г1. Якщо в ♀ Г2 в умовах АНМ зберігався баланс між активністю симпатичної та парасимпатичної ланок, то в ♀ Г1 в умовах АНМ переважала активність симпатичної ланки.*

Висновки. *Світлова депривація посилює симпатичні впливи на ритм серця в самців щурів і не змінює вихідного балансу активності симпатичної та парасимпатичної ланок в самиць. Розвиток адреналін-індукованого некрозу міокарда на тлі світлової депривації характеризується більшою, ніж за збереженого балансу день/ніч, активністю парасимпатичної ланки в регуляції ритму серця самців та викликає розвиток брадикардії. У самиць світлова депривація сприяє підтриманню балансу між активністю симпатичної та парасимпатичної ланок в умовах адреналін-індукованого некрозу міокарда, на відміну від самиць, що перебували в умовах зміни циклу день/ніч і демонстрували посилення активності симпатичної ланки АНС при розвитку некрозу міокарда.*

КЛЮЧОВІ СЛОВА: **некроз міокарда; варіабельність серцевого ритму; світлова депривація, стать**

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