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THE ROLE OF PEPSINOGENES AND SOME INTESTINAL HORMONES IN PATHOGENESIS OF GASTROESOPHAGEAL REFLUX DISEASE

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Abstract

80 patients with gastroesophageal reflux disease (GERD) were examined. The diagnosis of GERD was based on the history of the disease, complaints of patients, the results of daily monitoring of pH in the lower third of the esophagus, data of fibroesophagogastroduodenoscopy, chromoendoscopy, ¹³C-octanoic breath test, gastrin-17 (G-17) concentration, pepsinogens I and II (P I and II) and cholecystokinin-pancreozymin (C-P) in serum.

After 24 hours pH monitoring of the lower third of the esophagus in 40 patients with GERD, the predominance of acid was found, and the other 40 patients had mixed refluxes. In patients with predominance of acid reflux, the mean values of half-life of solid food evaluation (T_{1/2}) according to ¹³C-octanoic breath test was (45.25±1.34) min. With the predominance of mixed refluxes in patients, there was a tendency towards hypokinetic motility of the stomach, indicating a slowdown in half-life of solid food evaluation from the stomach up to (139.24±11.87) min. With the predominance of acid reflux also was observed a significant reduction in the concentration of G-17 and C-P, an increase in P I and a decrease in the concentration of P II. Hypergastrinemia, high levels of C-P and P II (37.44±3.41) µg/l (p<0,05) were diagnosed with the predominance of mixed refluxes.

Keywords: gastroesophageal reflux disease, 24 hours pH monitoring of the lower third of the esophagus, ¹³C-octanoic breath test, gastropanel, cholecystokinin-pancreozymin.

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1. Introduction

Gastroesophageal reflux disease (GERD) is a multifactorial disease, the occurrence of which is important as a contributing factor, among which stress, work associated with inclining, obesity, smoking, some medication, nutrition, and determinative factors, which include cardiac failure, reflux of gastric and duodenal contents, decreased esophageal clearance and resistance of the mucous membrane of the esophagus [1, 2].

A significant number of exogenous and endogenous factors influences the tonus of the lower esophageal sphincter [3, 4]. In particular, the pressure in it decreases under the influence of a number of gastrointestinal hormones: glucagon, gastrin, somatostatin, cholecystokinin, secretin, vasointestinal peptide, enkephalin [5, 6]. Reducing the tonus of the lower esophageal sphincter may also be due to a violation of receptor sensitivity to gastrin, both at the periphery and in the central nervous system [7, 8]. Due to the violation of regulatory mechanisms that coordinate the periodic motor activity of the gastrointestinal tract, there is a disintegration of the secondary peristalsis of the esophagus, dysfunction of the lower esophagus and pyloric sphincter, increasement of intragastric and intraduodenal pressure [9].

2. Aim of research

To study the nature of changes in the motor-evacuation function of the stomach and indicators of 24 hours pH monitoring in the lower third of the esophagus, to establish the role of gastrin-17, cholecystokinin-pancreozymin, changes in the level of pepsinogens and their correlation in the pathogenesis of gastroesophageal reflux disease.

3. Materials and methods

The work was performed at the Department of general practice (Family medicine), physical rehabilitation and sports medicine of the Ivano-Frankivsk National Medical University, for the period 2014–2016. 80 patients with GERD were examined. The age of the patients varied from

19 to 73 years and amounted to an average of 45.88 ± 1.32 years, the mean duration of GERD was 6.67 ± 1.07 years.

The main verifying method in the diagnosis of GERD is fibroesophagogastroduodenoscopy (FEGD) according to the generally accepted method using the “Olympus” apparatus GIF-XPE. In establishing the clinical diagnosis of GERD, the degree of damage to the esophagus was determined after an endoscopic examination (A, B, C, D), according to the Los Angeles classification [10]. Among the additional endoscopic diagnostic methods, the most accessible and informative was the chromoscopy with the use of dye (Lugol solution) [11].

The statistical processing of the obtained results was carried out using the software – Microsoft Excel spreadsheet and application package Statistica v. 10.0 StatSoft, USA. Estimation of the probability of divergence of mean values of the conducts using the dual t-criterion of Student.

Experimental procedures

Daily (24-hour) esophageal pH. The method allowed to quantify the frequency, duration and daily dynamics of gastroesophageal and gastroduodenal reflux, the number and duration of episodes of $\text{pH} < 4$ and > 7 in the esophagus. Lower esophageal pH monitoring in the night and day periods was performed by acidogastrograph AG-1rN-M. The night period was determined from the time when the patient lay in bed to fall asleep until the morning when he woke up. In the analysis of daily monitoring of pH in the lower third of the esophagus, we evaluated the measurements of the DeMeester scale.

The motor-evacuation function of the stomach was examined using the ^{13}C -octanoic breath test. The air analysis was carried out on IRIS (Wagner-Analysen-Technic, Germany) infrared analyser. A concentration curve of $^{13}\text{CO}_2$ was constructed and the half-life of solid food from the stomach in minutes ($T_{1/2}$) and the drainage factor (GEC) were calculated.

Research of the concentration of gastrin, pepsinogen I and II in the blood was carried out on the basis of the GasroPanelBIOHIT (Finland) test system. The basis of the technology of the panel “GasroPanel” is the principle of enzyme-linked immunosorbent assay (ELISA). The special ELISA equipment of the company “VIONIT” allowed to measure the level of pepsinogens I and II, gastrin-17. Investigation of the concentration of cholecystokinin-pancreozymin in serum was performed by the enzyme-linked immunosorbent method, using “Peninsula Laboratories Inc” (USA) and expressed in ng/ml.

4. Results

Young patients (16-29 years of age) were more likely to have an A-B degree of esophagitis than C-D ($\chi^2=2.571$; $p>0.05$), while people over 60 years of age were more likely to have a degree of C-D (in 3 out of 5 cases; $\chi^2=0.171$; $p>0.05$). At the age of 30-59, B than A, C and D was significantly more likely to be diagnosed ($\chi^2=6.332$; $p<0.05$; $\chi^2=3.741$; $p>0.05$; $\chi^2=12.692$; $p<0.001$). At the duration of GERD 1–5 years, the stages A (10.00 %) and B (5.00 %) – $\chi^2=4.000$ were significantly more often diagnosed; $p<0.05$. At the duration of the disease up to 10 years, a tendency towards the progression of the esophagitis is noted: stage B is diagnosed at 22,50 %, and C – at 17,50 %, which has been significantly more developed in compared to the duration of the disease to 5 years ($\chi^2=10.329$; $p<0.01$). In patients with a long history of GERD (>10 years), the stages of C-D ($\chi^2=8,526$; $p<0.01$) were significantly more often established.

Two cases of Barrett’s esophagus were detected and histologically verified with the chromoendoscopy. These patients were subsequently excluded from the study.

After 24-hours pH monitoring in the lower third of the esophagus in 40 patients with GERD, the predominance of acid was found, and in the other 40 patients were detected mixed refluxes.

The peculiarities of the daily pH analysis with the predominance of acid refluxes were: the mean value of the intra-esophageal pH (4.58 ± 0.33) (pH min – pH max, respectively, 1.64 ± 0.23 – 6.45 ± 0.52), indicating about a stable acid aggression of the lower third of the esophagus. In 77.50 % of patients, the persistent predominance of acid reflux with average values (3.22 ± 0.15) was established. At 15.00 %, respectively, the average values of the intra-vascular pH were (4.78 ± 0.36), and 7.50 % – (7.08 ± 0.42). The average time of throwing aggres-

sive gastric juice was 1 hour per day (77.24 ± 5.14) min. The average number of acid episodes of >5 min was 57.

Often, acidic refluxes were recorded in the first half of the night (7.28 ± 0.64). In the upright position of the body, the acidity of the lower third of the esophagus was observed during (8.24 ± 0.75) min, often these episodes of long-term acid reflux were observed after ingestion of fat or use of large portions of food. In patients with predominance of acid reflux, the DeMeester index was (28.78 ± 0.18) (normally up to 14.72).

With the predominance of mixed reflux in patients with GERD, the mean intra-invasive pH value with a tendency toward neutral indices (5.69 ± 0.29) was statistically significant ($p < 0.001$). The variation of the minimum and maximum values of the esophagus pH was (3.01 ± 0.12) – (7.89 ± 0.66), respectively.

Such values are evidently due to the presence of duodenal and biliary content in the esophagus. Total daily time, when in the lower third of the esophagus, the pH < 4.0 was less than an hour (44.84 ± 2.07) min ($p_1 < 0.01$), however, the time of contact of the lower third of the esophagus with alkaline contents of the pH > 7.0 (75.25 ± 0.61) min ($p_1 < 0.001$). The total DeMeester mean was (18.87 ± 0.09) ($p_1 < 0.01$).

According to the results of the ^{13}C -octanoic breath test in the examined patients, in the practically healthy people, the half-life of food ($T_{1/2}$) from the stomach was (64.69 ± 2.17) min, and the drainage factor (GEC) – (3.97 ± 0.03). In patients with predominance of acid reflux, according to daily monitoring of pH in the lower third of the esophagus, the mean values of the ^{13}C -octanoic breath test were $T_{1/2}$ – (45.25 ± 1.34) min, $p < 0.05$ and GEC (4.15 ± 0.13 , $p < 0.05$, indicating the predominance of the hypermotor type of gastric contractility and excessively accelerated evacuation of food.

With the predominance of mixed refluxes in patients, there was a tendency toward hypokinetic motility of the stomach, indicating a slowdown $T_{1/2}$ to (139.24 ± 11.87) min, as compared with healthy ($p < 0.05$), and with patients in which determined the dominance of acid refluxes ($p_1 < 0.05$), and a significant decrease in GEC – (2.12 ± 0.19) ($p < 0.05$; $p_1 < 0.05$).

All patients with GERD have been tested for the content of gastrin-17, cholecystokinin-pancreozymin, pepsinogen-I and pepsinogen-II [12]. They have a multidirectional change in hormonal secretion and motor-evacuation function of the lower part of the esophagus, stomach and sphincter apparatus.

With the predominance of acid reflux, a significant reduction in the concentration of T-17 with mean values (4.28 ± 0.21) pmol/l ($p < 0.05$) and C-P (3.25 ± 0.17) ng/ml ($p < 0.05$), a rise in P I to (375.18 ± 15.25) $\mu\text{g/l}$ ($p < 0.05$), and a decrease in the concentration of P II to (15.12 ± 1.24) $\mu\text{g/l}$ ($p > 0.05$) and increase the ratio of P I/P II to (22.83 ± 1.89) of cond. units. Instead, with the predominance of mixed reflux, hypergastrinemia (24.28 ± 1.87) pmol/l ($p < 0.05$), high level of C-P (7.27 ± 0.42) ng/ml ($p < 0.05$) and PII (37.44 ± 3.41) $\mu\text{g/l}$ ($p < 0.05$). In these patients, the concentration of P I tended to decrease but did not significantly differ from healthy subjects (112.27 ± 8.74) $\mu\text{g/l}$ ($p > 0.05$), although there was a significant decrease in the P I / P II ratio to (5.57 ± 0.47) of the cond. units ($p < 0.05$).

5. Discussion

In patients with predominance of acid reflux, the accelerated evacuation of the stomach is likely to indicate a dyskinetic prolapse and propulsion through the lower diaphragmatic sphincter and the ingestion of aggressive acidic contents in the stomach.

In 70 % of patients with GERD with predominance of acid reflux hypertonic-hyperkinetic type of motor-evacuation function of the stomach is noted. This is also evidenced by the presence of a direct strong correlation between $T_{1/2}$ and the pH mean in the lower part of the esophagus ($r = 0.85$; $p < 0.001$) and GEC pH ($r = 0.79$; $p < 0.001$). Normal kinetic and hypokinetic motility of the stomach was observed rarely. Therefore, it should be considered that in patients with a combination of hyperkinetic state of motility and prolonged acid reflux in the esophagus, the acid component itself plays a leading role in the progression of the disease, which causes the need for higher doses of antacids. Instead, according to the results of our own studies, it was found that in patients with frequent and prolonged alkaline reflux, the hypotonic-hypokinetic type (57.5 %) was characteristic, as evidenced by the presence of the average strength of a direct correlation between $T_{1/2}$ and the

prevalence of alkaline esophagitis pH ($r=0.62$; $p<0.01$) and GEC and pH ($r=0.68$; $p<0.01$). Also, one out of five patients did not have a significant gastrointestinal motility disorder. In our opinion, structural changes in SOS are due to the damaging effects of the acidic component and the long stasis in the stomach and the remitting of retrograde duodenal contents containing bile acids. Therefore, before treatment in such patients, it is appropriate to attach a prokinetic drug and an agent that would neutralize the biliary component of the reflux.

After analysis of the correlation of blood T-17 concentration with the 24-hours pH monitoring of the lower part of the esophagus using the odds ratio method, it was found that in patients with low levels, acid reflux with pH ranged from 3.0 to 3.99 (OR=8.69; $p=0.04$) and with pH 2.0–2.99 (OR=7.43; $p=0.02$) and somewhat less frequently from pH 1.0–1.99 (OR=3.0; $p=0.03$). (not indicated in the presented article). Most often (23.4 ± 2.4) cases in the lower third of the esophagus were recorded pH in the range of 2.0–2.99, less (15.4 ± 1.6) cases per day - with pH 3.0-3.99. In patients with GERD in (8.9 ± 1.9) cases per day, reflux was found to be too acidic with pH in the range of 1.0–1.99. The alkaline reflux in patients with GERD with a low level of gastrin in serum is not registered.

In subjects with elevated level G-17, the results of daily monitoring of the pH of the lower third of the esophagus were more often recorded alkaline reflux with pH in the range of 7.0–7.99 (OR=2.6; $p=0.04$), the total number of which was (15.5 ± 2.1) episodes per day ($p<0.05$), whereas at normal level of T-17 only in (2.3 ± 0.4) cases. Also in patients with GERD with high level of T-17 blood in (6.2 ± 1.1) cases in the lower third of the esophagus, the pH was determined in the range of 8.0–8.5, in the absence of episodes of such ingestion in patients with normal and lowered level G-17. This fact can be explained by the stimulating effect of gastrin on peristalsis of the gastrointestinal tract. G-17 induces secretin secretion, and C-P, consequently, enhances the secretion of bile and pancreatic secretion that is thrown into the stomach and esophagus. C-P also stimulates the secretion of the gastric secretion, suppresses its evacuation, and relaxes the Oddi sphincter.

After analysing the relationship between the level of T-17 in the blood, the duration of GERD, it was found that in patients with a long history of disease to one and from one to five years of age, its serum concentration did not differ and equalled, respectively (5.41 ± 1.12) pmol/l and (7.33 ± 1.40) pmol/l ($p>0.05$). In 75.50 % of patients with GERD with a history of the disease >5 years, the concentration of G-17 was (20.55 ± 3.10) pmol/l, exceeding the indexes of the first duration of the disease by 3.8 and 2.8 times ($p_{1,2}<0.05$).

The emergence of pathological gastroesophageal reflux is largely dependent on the disturbed motility of the stomach and duodenum [13, 14], while the infection and eradication of *Helicobacter Pylori* do not play an important role in the genesis of GERD, which is also noted in the 4 Maastricht consensus. In addition, the discoordination of the motor-evacuator function of the upper parts of the gastrointestinal tract, in particular the stomach, is the basis for the appearance of mixed (duodenal refluxes), due to the imbalance in the contractile activity of the antral and pyloric divisions and the duodenum [15, 16]. The rate of reduction of the stomach also depends on the level of some gastrointestinal hormones, in particular, ghrelin (a direct biochemical predictor of this process) and gastrin [17, 18]. Reducing the tone of the stomach and slowing the evacuation period from it is often combined with the increased contractile capacity of the duodenum and the appearance of duodeno-gastric and gastroesophageal reflux and the development of GERD [19, 20]. In addition, there is a certain correlation between the appearance of such refluxes with the function of gastric receptors, especially as gastrin enhances motor function of the stomach.

6. Conclusions

1. In gastroesophageal reflux disease, A (23.75 %), B (37.50 %), and C (25.00 %) degrees of esophagitis, according to the Los Angeles Endoscopic Classification, were predominantly diagnosed.

2. According to 24-hours pH monitoring of the lower third of the esophagus, patients with gastroesophageal reflux disease with almost identical frequency recorded acid and mixed reflux. The number of episodes of acid regurgitation was 57 cases, the retention time of $pH<4$ – (77.24 ± 5.14) min per day. The frequency of reflux mixed with a $pH>7$ was 9 cases, respectively, and their duration – (75.25 ± 0.61) min per day.

3. In cases of prevalence of acidic esophageal reflux, an increase in contractional function of the stomach was observed with $T_{1/2}$ (45.25 ± 1.34) min ($p < 0.05$), and in case of mixed reflux there was a slowed-down type of $T_{1/2}$ (139.24 ± 11.87) min ($p < 0.05$).

4. In patients with gastroesophageal reflux disease with a low level of gastrin-17 in serum, acidic reflux was found to prevail from pH 3.00–3.99 (OR=8.69; $p=0.04$), pH 2.00–2.99 (OR=7.43; $p=0.02$), and pH 1.00–1.99 (OR=3.0; $p=0.03$), and for increasing its concentration, mixed reflux was recorded with pH at intervals of 7.00–7.99 (OR=2.6, $p=0.04$).

5. In patients with gastroesophageal reflux disease, with the predominance of acid reflux, the concentration of pepsinogen I in the blood was 3.5 times higher than in healthy subjects, and the level of pepsinogen II did not differ from the norm with increasing their ratio, and with the predominance of mixed – the concentration of pepsinogen I in the blood was within the normal range with an increase in the level of pepsinogen II to (37.44 ± 3.41) $\mu\text{g/l}$ and a decrease in their ratio to (5.57 ± 0.47) cond. unit.

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RISK FACTORS AND COMORBIDITY IN PATIENTS WITH ATRIAL FIBRILLATION AND ISCHEMIC HEART DISEASE

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Abstract

The aim of the study was to evaluate the risk factors and the incidence of comorbidity in patients with coronary heart disease, depending on the presence of atrial fibrillation.

Materials and methods of research: a retrospective analysis of 222 histories of illnesses of patients with coronary heart disease who undergo inpatient treatment, aged from 39 to 88 years, has been conducted. Depending on the presence of atrial fibrillation, all patients were divided into 2 groups: group 1 (main) – patients with ischemic heart disease with atrial fibrillation (n=105), group 2 (comparison) – patients with ischemic heart disease without atrial fibrillation (n=117).

Results. In the group of patients without AF, the proportion of persons with inherited exacerbations of IHD was 64.29 %, while in the main group – 25.0 %, the differences did not reach the statistically significant level, but this relationship is confirmed by the results of the rank correlation analysis – between the presence AP and heredity revealed a significant weak feedback – $c=-0.21$ ($p<0.05$). The diseases that were observed in the examined patients with coronary artery disease present acute stroke, angina pectoris, acute myocardial infarction, hypertension, diabetes, pathology of the kidneys and the thyroid gland, diastolic dysfunction and obesity. The groups differed in the proportion of patients with stroke – in the group with AF, it was significantly ($p=0.002$) higher – 23.81 %, in compare to 8.55 % in the comparison group.

Conclusions: The presence of atrial fibrillation in patients with coronary heart disease is associated with a high degree of comorbidity. First of all, with the combination of IHD and atrial fibrillation, a high incidence of hypertension, diabetes mellitus, obesity, acute stroke, kidney disease and thyroid gland is established.

Keywords: atrial fibrillation, ischemic heart disease, comorbid pathology, risk factors.

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1. Introduction

At present, atrial fibrillation (AF) is one of the most common forms of arrhythmias, which often leads to acute cerebrovascular disruption and has a negative social significance [1, 2].

According to statistics, every fifth case of stroke is due to the presence of atrial fibrillation [3, 4]. In this case, ischemic strokes in this type of arrhythmias often have fatal consequences, and those surviving patients have consequences with severe disability [5].

It is known, that spread of comorbidity is connected with age [6]. According to literature, with age, the prevalence of atrial fibrillation is also increasing: almost 10 % of people over the age of 80 undergo AF; for those who have reached the age of 94 this figure is up to 25 % [7, 8]. The study of risk factors and comorbidity in patients with atrial fibrillation of non-valvular genesis requires a detailed analysis, since it has clinical significance for the prevention and treatment of this category of patients.

2. Aim of the research

To evaluate the risk factors and the incidence of comorbidity in patients with ischemic heart disease, depending on the presence of atrial fibrillation.

3. Materials and methods of the research

A retrospective analysis of 222 histories of illnesses of patients with ischemic heart disease (IHD) undergoing in-patient treatment on the basis of the “Regional Clinical Hospital named after I. I. Mechnikov” during 2015–2016, aged from 39 to 88 years.

Depending on the presence of atrial fibrillation, all patients were divided into 2 groups:

Group 1 (main) – patients with ischemic heart disease with atrial fibrillation (n=105).

Group 2 (comparison) – patients with ischemic heart disease without atrial fibrillation (n=117).

From all patients were collected anamnestic data, physical and laboratory-instrumental tests (clinical and biochemical blood tests, general urine analysis, electrocardiogram registration, radiography of the chest, ultrasound examination of the abdominal cavity). All patients were measured anthropometric data with the calculation of the body mass index (BMI) according to the standard formula [9]. To evaluate the function of the kidneys were performed the determination of creatinine and the calculation of glomerular filtration rate (GFR) according to the MDRD formula [10] according to the recommendations of the American National Federation of Nephrology [5, 10]. In order to evaluate the hemodynamic parameters, patients underwent pre-pleural cardiac examination of the heart using the standard method [9]. In the presence of indications, the patient was monitored with the Holter's method. The functional state of the thyroid gland was determined by the evaluation of thyroid hormones with the subsequent consultation of the endocrinologist.

For statistical processing of the research material, the following biostatistical methods were used: verification of the normality of the distribution of quantitative attributes in separate groups of comparison by the Shapiro-Wilks criterion; estimation of the reliability of the mean difference for quantitative signs with abnormal distribution by the Mann-Whitney criterion (U); correlation analysis with calculation of Spearman rank correlation coefficients (ρ). To describe the selective abnormal distribution of quantitative signs, the median (Me) and the interquartile scale (25 %, 75 %) were used. For the calculated relative values (P), the average error of the relative magnitude (mR) and 95 % confidence intervals were determined by the Wilson method with the correction for continuity. For the analysis of the relationships between different features, a correlation analysis was used to calculate the Spearman rank correlation coefficients (ρ) [11].

The critical value of the statistical significance level when checking the null hypotheses was taken as 0.05 (5 %). When the value of $p > 0.05$ was obtained, the difference between the indices was considered to be unreliable [12].

Statistical processing of the results was performed using the STATISTICA 6.1 software (StatSoft Inc., Serial number AGAR909E415822FA), Excel 2010, and the MedCalc Statistical Software trial version 17.4 software package. (MedCalc Software bvba, Ostend, Belgium).

4. Results of the research

The general characteristics of the study groups are shown in **Table 1**.

Since the abnormal distribution of quantitative signs was observed in the overwhelming majority (over 80 %) of indicators for describing the central trend of data, the median and the interquartile Me (25 %, 75 %) were used.

Analysis on the homogeneity of the study groups showed that there were no significant differences in the distribution of patients in groups depending on sex, anthropometric indicators (average height, weight, BMI), risk factors (heredity, smoking) ($p > 0.05$), indicating their comparability and allows comparisons of other parameters.

The presence of statistically significant differences according to age characteristics was determined ($p < 0.001$): in the group with AF, on average, older patients (average age 65.0 (59.0; 72.0)) compared with the group without AF (60.0 (53.0; 69.0)). The proportion of persons over the age of 60 in the main group is 68.57 %, in the comparison group – 48.72 % ($p = 0.003$). The inverse weak link between the presence of AF (group) and age is the Spearman correlation coefficient $c = -0.23$ ($p < 0.05$).

The proportion of persons with BMI greater than 30 is higher in the group without atrial fibrillation (36.46 % versus 27.27 % in the AF group) without statistically significant differences.

In the group of patients without AF, the proportion of persons with inherited exacerbations of IHD was 64.29 %, while in the main group – 25.0 %, the differences did not reach the statistically significant level, but this relationship is confirmed by the results of the rank correlation analysis – between the presence AF and heredity revealed a significant weak feedback – $c = -0.21$ ($p < 0.05$).

The diseases observed in the examined patients with ischemic heart disease are acute stroke, angina pectoris, acute coronary syndrome (ACS), hypertension (HT), diabetes mellitus (DM), kidney and thyroid pathology, diastolic dysfunction (DD) and obesity (**Table 2**).

Table 1

The general characteristics of the study groups with IHD

Indicators	Totally observed	Group with AF	Group without AF	p
Number	222 (100.0 %)	105 (47.3 %)	117 (52.7 %)	—
Sex, n (%)				
man	55 (52.38 %)	59 (50.43 %)	59 (50.43 %)	0.771*
female	50 (47.62 %)	58 (49.57 %)	58 (49.57 %)	
Age				
Min – max	39.0–88.0	42.0–88.0	39.0 – 84.0	—
Average age Me (25 %; 75 %)	64.0 (55.0; 70.0)	65.0 (59.0; 72.0)	60.0 (53.0; 69.0)	<0.001 [#]
Structure by age, n (%)				
Age<60 years	93 (41.89 %)	33 (31.43 %)	60 (51.28 %)	0.003*
Age>60 years	129 (58.11 %)	72 (68.57 %)	57 (48.72 %)	
Anthropometric indicators, Me (25 %; 75 %)				
Height (cm)	170.0 (162.0; 178.0)	170.0 (160.0; 180.0)	168.0 (163.0; 176.0)	0.446 [#]
Weight (kg)	82.0 (70.0; 97.0)	81.0 (70.0; 95.0)	82.5 (72.0; 98.0)	0.492 [#]
BMI				
Min – max	19.53–47.9	19.53–42.2	20.4–47.9	—
Average BMI Me (25 %; 75 %)	29.05 (23.88; 33.31)	28.55 (23.44; 32.05)	29.48 (24.79; 34.40)	0.146 [#]
BMI>30, n (%)	53 (23.87 %)	18 (17.14 %)	35 (29.91 %)	0.026*
Risk factors, n (%)				
n (data available)	26	12	14	
Heredity (data available)	12 (46.15 %)	3 (25.0 %)	9 (64.29 %)	0.053**
Heredity (observed)	12 (5.41 %)	3 (2.86 %)	9 (7.69 %)	0.097**
n(data available)	20	8	12	
Smoking (data available)	10 (4.5 %)	2 (1.9 %)	8 (6.84 %)	0.072**
Smoking (observed)	10 (4.5 %)	2 (1.9 %)	8 (6.84 %)	0.077**

Note: p – differences between groups; # – for quantitative attributes according to the Mann-Whitney criterion; * – for qualitative characteristics according to criterion χ^2 of Pearson; ** – for qualitative signs according to Fischer's exact criterion

The groups differed in the proportion of patients with acute stroke – in the group with AF, it was significantly ($p=0.002$) higher – 23.81 %, compared with 8.55 % in the comparison group. Also, the differences were determined by angina – in the group without AF, the proportion of patients with coronary artery disease was higher and amounted to 58.97 %, whereas among patients with atrial fibrillation – 31.43 % ($p<0.001$). The difference is mainly due to discrepancies in the presence of angina of the 3rd functional class – 38.46 % in the group without AF and 20.95 % in the presence of atrial fibrillation ($p=0.005$).

Table 2
Comorbidity in patients with ischemic heart disease, n (%)

Disease	Totally observed (n=222)	Group with AF (n=105)	Group without AF (n=1117)	p*
1	2	3	4	5
Acute stroke, n (%)				
without acute stroke	187 (84.23 %)	80 (76.19 %)	107 (91.45 %)	0.002
with acute stroke	35 (15.77 %)	25 (23.81 %)	10 (8.55 %)	
ACS, n (%)				
without ACS	176 (79.28 %)	87 (82.86 %)	89 (76.07 %)	0.213
with ACS	46 (20.72 %)	18 (17.14 %)	28 (23.93 %)	
Angina pectoris, n (%)				
without angina pectoris	120 (54.05 %)	72 (68.57 %)	48 (41.03 %)	<0.001
with angina pectoris	102 (45.95 %)	33 (31.43 %)	69 (58.97 %)	
Functional class of angina pectoris, n (%)				
2	29 (13.06 %)	11 (10.48 %)	18 (15.38 %)	0.279
3	67 (30.18 %)	22 (20.95 %)	45 (38.46 %)	0.005
4	2 (0.9 %)	0 (0 %)	2 (1.71 %)	0.534
Hypertension (HT), n (%)				
without hypertension	24 (10.81 %)	15 (14.29 %)	9 (7.69 %)	0.114
with hypertension	198 (89.19 %)	90 (85.71 %)	108 (92.31 %)	
Degree of HT, n (%)				
2	131 (59.01 %)	59 (56.19 %)	72 (61.54 %)	0.419
3	67 (30.18 %)	31 (29.52 %)	36 (30.77 %)	0.883
Stage of HT, n (%)				
1	11 (4.95 %)	8 (7.62 %)	3 (2.56 %)	0.083
2	118 (53.15 %)	57 (54.29 %)	61 (52.14 %)	0.749
3	70 (31.53 %)	25 (23.81 %)	45 (38.46 %)	0.019
Diabetes mellitus (DM), n (%)				
n	211	102	109	0.564
without DM	124 (58.77 %)	62 (60.78 %)	62 (56.88 %)	
with DM	87 (41.23 %)	40 (39.22 %)	47 (43.12 %)	
Decrease of GFR, n (%)				
n	181	79	102	0.400
without pathology	54 (29.83 %)	21 (26.58 %)	33 (32.35 %)	
with pathology	127 (70.17 %)	58 (73.42 %)	69 (67.65 %)	

Continuation of Table 2

1	2	3	4	5
Hypo- or hyperthyroidism, n (%)				
n	181	75	106	
without pathology	86 (47.51 %)	29 (38.67 %)	57 (53.77 %)	0.045
with pathology	95 (52.49 %)	46 (61.33 %)	49 (46.23 %)	
Obesity, n (%)				
n	166	67	99	
without obesity	90 (54.22 %)	39 (58.21 %)	51 (51.52 %)	0.396
with obesity	76 (45.78 %)	28 (41.79 %)	48 (48.48 %)	
Stage of obesity, n (%)				
0	90 (54.22 %)	39 (58.21 %)	51 (51.52 %)	0.396
1	45 (27.11 %)	20 (29.85 %)	25 (25.25 %)	0.711
2	21 (12.65 %)	4 (5.97 %)	17 (17.17 %)	0.002
3	9 (5.42 %)	4 (5.97 %)	5 (5.05 %)	0.730
4	1 (0.6 %)	0 (0 %)	1 (1.01 %)	0.323
Diastolic dysfunction (E/A<1), n (%)				
n	143	40	103	
without DD	62 (43.36 %)	31 (77.5 %)	31 (30.1 %)	<0.001
with DD	81 (56.64 %)	9 (22.5 %)	72 (69.9 %)	

Note: * – *p* is the difference between the groups according to Pearson's criterion χ^2 , including the Yeats correction

Patients with atrial fibrillation did not differ in the proportion of people with acute myocardial infarction – in general, 20.72 % with a minor excess in the comparison group (23.93 % vs. 17.14 %), hypertension (92.31 % in the group without AF, and 85.71 % in the AF group), diabetes mellitus (43.12 % and 39.22 % respectively), kidney pathology (67.65 % and 73.42 % respectively), obesity (48.48 % and 41.79 % respectively).

In the structure of the distribution according to the HT stage, there were no discrepancies regarding the stage of the disease, and then differences were observed only in the proportion of patients with 3rd stage HT, which was higher in the comparison group (38.46 % in the group without AF with 23.81 % with atrial fibrillation).

It should be noted that there are significant differences ($p=0.002$) between the groups in the proportion of patients with obesity of the 2nd stage – the proportion of such patients is greater in the group without AF (5.97 %), compared with the group with AF (17.17 %).

The proportion of patients with diastolic dysfunction (DD) in the group without AF is significantly higher (69.9 %) compared with the group without AF – 22.5 % ($p<0.001$).

Thyroid dysfunction was found in the main group with a frequency of 61.33 % (95 % CI 50.02–71.54), which is statistically significant ($p<0.05$) and can be compared more with the group without AF – 46.23 % (95 % CI 37.03–55.68). In addition to the thyroid pathology, in the main group, the frequency of GPMC was significantly higher ($p<0.05$), significantly lower – the frequency of angina and DD (**Fig. 1**).

In the main study group, the incidence of concomitant pathology in the order of decline predominated: HT, kidney pathology and thyroid pathology; whereas in the comparison group – HT, diastolic dysfunction and kidney pathology. The last ranked place in the frequency of occurrence in the group with AF was acute myocardial infarction – 17.14 % (95 % CI 11.13-25.48), while in the comparison group, the penultimate one with a frequency of 23.93 % (95 % CI 17, 11–32.41).

By rank correlation analysis, reliable ($p < 0.05$) inverse correlation bonds of mean strength of AF with diastolic dysfunction ($\rho = -0.43$; $p < 0.001$) were found; weak – with angina pectoris ($\rho = -0.28$; $p < 0.001$) and degree of GC ($\rho = -0.20$; $p = 0.003$). Consequently, in the presence of these diseases, atrial fibrillation develops less frequently. Direct weak correlations were detected between AF and acute stroke ($\rho = 0.21$; $p = 0.002$) and pathology of the thyroid gland ($\rho = 0.15$; $p = 0.045$), therefore, atrial fibrillation develops more frequently in these pathologies.

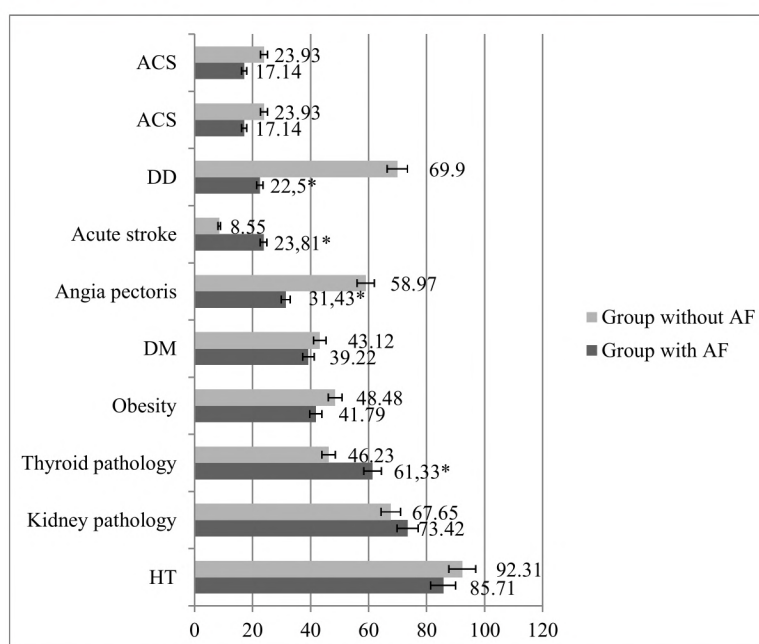


Fig. 1. Comorbidity in the examined patients with coronary artery disease, depending on the presence of atrial fibrillation ($P \pm m$, %): * – $p < 0.05$ compared with the group without AF by the Pearson χ^2 criterion

5. Discussion

According to the results of numerous studies with age, increases the incidence of cardiovascular diseases such as hypertension, ischemic heart disease, heart failure, various cardiac arrhythmias, post-infarction cardiosclerosis [13, 15].

The results of the study are consistent with the literature, which indicates that the presence of atrial fibrillation also increases with age [15, 16]. At the same time, according to modern ideas and results obtained, the incidence of atrial fibrillation and the frequency of comorbidity [17] increases with age, which greatly complicates the diagnosis and choice of therapeutic tactics when combined with a patient with comorbidity and atrial fibrillation [18].

Nowadays, there are data on the relationship between body mass and the development of cardiovascular disease [19]. Some studies indicate that patients with high body weight have lower mortality in patients with heart failure than patients with low body weight [9, 20]. According to the European guidelines, in the presence of atrial fibrillation, the mass of the body may be a predictor of its development [7, 21], which does not contradict the results. According to the study, the incidence rate of obesity was found to be rather high in both groups.

Identification at an early stage the diagnosis, the patient's risk factors and comorbid pathology is important for the primary prevention as well as for secondary prevention in order to prevent the development of repeated acute stroke, which often develop in this category of patients

[22, 23]. Development of individual practical recommendations for the diagnosis and treatment of atrial fibrillation in patients with ischemic heart disease, depending on the presence of comorbidity pathology [24], which is planned in the future, has a promising value in practice.

6. Conclusions

1. The presence of atrial fibrillation in patients with coronary heart disease is associated with a high degree of comorbidity that increases with age.

2. In case of combination IHD and atrial fibrillation was detected a high incidence of hypertension, diabetes mellitus, obesity, acute cardiovascular disorders, kidney disease and thyroid gland.

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ANALYSIS OF COMPLICATED CERVICAL ECTOPY CLINICAL COURSE AND RECURRENCE

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Abstract

Physiological cervical ectopy (CE) is commonly found in young women, during pregnancy or intake of oral contraceptives. The complicated (pathological) CE, particularly in conditions of vaginal microbiota disturbances and human papillomavirus (HPV) infection, requires intervention, including optimal treatment and effective relapse prevention approaches.

Aim. The aim of the research was to investigate the complicated CE clinical course and recurrence features on the basis of a retrospective analysis of archival medical records in order to optimize the tactics of the complicated CE management and the relapse prevention measures.

Materials and methods. In the observational cross-sectional retrospective study using the continuous sampling method were included 740 case reports of women, who underwent inpatient treatment of gynecological pathology in Lviv Municipal Clinical First Aid Hospital in 2006–2017. The inclusion criterion was the presence of firstly diagnosed or recurrent CE. Exclusion criteria: absence of CE colposcopic or morphological confirmation, presence of physiological CE (on conditions of cytological, bacterioscopic, bacteriological and colposcopic abnormalities absence). Subsequently, three groups were formed: control group (n=150) – healthy women, who applied for a regular gynecological examination; group 1 (n=483) – women with firstly diagnosed CE; group 2 (n=257) – women with recurrent CE. Differences in mean values were considered significant with a probability level of at least 95 % ($p < 0.05$).

Results. Patients with recurrent CE had more pregnancies ($p < 0.05$) than the ones with firstly diagnosed CE and women of control group. The proportion of women with high parity in group 1 and group 2 was three times higher ($p < 0.05$), than in group 3. The proportion of artificial abortions in patients of groups 1 and 2 was more than two times higher ($p < 0.05$), than in women in the control group, group 2 patients had more miscarriages and missed miscarriages ($p < 0.05$) in comparison to the control group. Menarche in patients with recurrent CE set in later in comparison to women of control group ($p < 0.05$). In group 2, the proportion of women with polymenorrhea was three times higher ($p < 0.05$), than in group 1 and control group respectively. The compromised gynecological history was more often ($p < 0.05$) detected in group 1 and group 2, than in the control group. In patients with recurrent CE a third of gynecologic diseases in the history (32.30 ± 2.92 %) and 23.60 ± 1.93 % – in patients with the firstly diagnosed CE were pelvic inflammatory diseases, significantly ($p < 0.05$) higher in comparison to the control group (3.33 ± 1.47 %). Almost half of patients in group 1 (48.25 ± 2.27 %) and group 2 (47.84 ± 3.13 %) had the history of frequent infectious diseases, this indicator was significantly ($p < 0.05$) higher in comparison to the control group (24.67 ± 3.52 %). In patients with recurrent CE chronic diseases of different etiology were diagnosed more often ($p < 0.05$) - their share made up 8.24 ± 1.72 %, while in patients with firstly diagnosed CE – 4.33 ± 0.92 %, in women of the control group – 1.33 ± 0.94 %. The firstly diagnosed CE was more frequent (in comparison to recurrent CE) ($p < 0.001$) characterized by asymptomatic course, whereas about half of patients with recurrent CE (46.69 ± 3.11 %) presented with different complaints. Cytological indicators of cervicitis were found more often in patients with firstly diagnosed and recurrent CE, than in women of control group, as well as vaginal candidosis ($p < 0.05$). Patients with recurrent CE more often ($p < 0.05$) underwent diathermoconization (24.12 ± 2.67 %) and cryodestruction of the cervix (8.17 ± 1.71 %) in comparison to patients of group 1. The most often performed treatment method in patients of both groups was diathermoconization. Treatment methods aimed to restore the hormonal balance, to normalize the state of vaginal microbiota were rarely found in both group 1 and 2, drugs that affect the reparation and regeneration of the epithelium have not been used in any case.

Conclusions. Recurrent symptomatic complicated CE should be interpreted as a complex problem that requires a multi-polar approach aimed at hormonal homeostasis, local immunity, and vaginal biocenosis normalizing. These measures should precede invasive treatment and further create optimal conditions for regeneration of the cervix.

Keywords: recurrent cervical ectopy, clinical course, treatment, prevention.

1. Introduction

The presence of columnar epithelium on the vaginal portion of the cervix is known as cervical ectopy (CE), it is often diagnosed in young nulliparous women aged under 25 (in 52.2–90 % of cases) [1]. CE found in adolescents and women of a young age, during pregnancy, intake of estrogen-containing oral contraceptives [2] is considered to be physiological, does not require any treatment and is subject to dynamic monitoring.

Subsequently, in women with CE under the influence of reproductive hormones the physiological process of metaplasia is activated and, as a result, the generative cells of the columnar epithelium are transformed into squamous epithelium by remodeling process, which involves active replication and cell differentiation [3].

The complicated (pathological) CE, particularly in conditions of vaginal microbiota disturbances and human papillomavirus (HPV) infection, requires intervention, especially taking into account the significant relapse rate, which has no tendency to decrease and reaches 40 % [4].

It has been established, that the presence of CE may increase the risk of sexually transmitted diseases (STDs) infection, in particular Chlamydia infection [5], Cytomegalovirus infection [6], HIV [7], and HPV [8].

Thus, the presence of active, long-term metaplastic changes creates favorable conditions for the HPV infection, its replication and persistence in the future [9, 10], which becomes especially relevant taking into account, that the HPV infection prevalence is the highest among women aged under 25–23.2 % [11]. In addition, in several cross-sectional studies, in particular among older women, a significant correlation between the presence of CE and the prevalence of HPV infection was found, in particular type 16 [12, 13] and even more frequent – type 18 [14], presence of which is known to be associated with the cervical adenocarcinoma. A number of recent studies have also found that vaginal biocenosis disturbances play an important role in the development of cervical squamous intraepithelial lesions [15, 16].

However, despite the fact that CE has been intensively studied in recent years, there is still no unanimous hypothesis explaining its etiopathogenesis, tactics of the complicated CE optimal treatment (in particular, in conditions of vaginal microbiota disturbances and HPV infection) and effective technologies of relapse prevention, which substantiates the relevance of further study of this problem.

2. Aim of research

The aim of the study is to investigate the complicated CE clinical course and recurrence features on the basis of a retrospective analysis of archival medical records in order to optimize the tactics of the complicated CE management and relapse prevention measures.

3. Materials and Methods

In the observational cross-sectional retrospective study using the continuous sampling method were included 740 case reports of women, who underwent inpatient treatment of gynecological pathology in Lviv Municipal Clinical First Aid Hospital in 2006–2017. The inclusion criterion was the presence of firstly diagnosed or recurrent CE.

Exclusion criteria: absence of CE colposcopic or morphological confirmation, presence of physiological CE (on conditions of cytological, bacterioscopic, bacteriological and colposcopic abnormalities absence).

Subsequently, three groups were formed:

- control group (n=150) – healthy women, who applied for a regular gynecological examination;
- group 1 (n=483) – women with firstly diagnosed CE;
- group 2 (n=257) – women with recurrent CE.

The following data was taken into account: age, obstetrical and gynecological history, extragenital pathology, complaints, laboratory diagnostic data (bacterioscopic, bacteriological, cytological, molecular (PCR) data) and colposcopy results.

Statistical processing of the study results was conducted using the Student's t-criterion. Differences in mean values were considered significant with a probability level of at least 95 % ($p < 0.05$).

4. Results

Firstly diagnosed CE was found in 65.27 ± 1.75 % (n=483) of patients, recurrent CE – in 34.73 ± 1.75 % (n=257) of patients.

The average age of women in the control group was 26.55 ± 0.64 years, in group 1 – 33.55 ± 0.58 years, in group 2 – 34.64 ± 0.57 years. In all groups, the prevalence of women of early and active reproductive age (18–34 years) was found: in group 1 this cohort of patients amounted to 60.31 ± 3.05 %, in the group 2 – 59.42 ± 2.23 %, whereas in the control group this indicator reached the value of 84.67 ± 2.94 %.

Every sixth patient with the firstly diagnosed CE (15.32 ± 1.64 %) and recurrent CE (15.56 ± 2.26 %) at least once underwent inpatient treatment because of the threatened miscarriage or premature labor. In every fifth patient (21.74 ± 1.88 % and 22.96 ± 2.62 % respectively), the complicated course of at least one pregnancy was diagnosed. Cervical rupture during the labor was diagnosed in 7.87 ± 1.23 % of patients in group 1 and in 10.89 ± 1.94 % of group 2 patients.

Menarche in patients with recurrent CE set in significantly ($p < 0.05$) later (on average in 13.59 ± 0.05 years) in comparison to women of control group.

The disorders of menstrual function were detected in more than half of women of all study groups (Table 1).

Table 1

Peculiarities of obstetrical and gynecological history of patients with CE in comparison to control group

Indicator	Group 1 (n=483)	Group 2 (n=257)	Control group (n=150)
Parity:	1.80 ± 0.06	$2.03 \pm 0.09^{***}$	$1.16 \pm 0.09^{* **}$
– labors:	1.32 ± 0.05	1.46 ± 0.06	$0.78 \pm 0.06^{* **}$
0, %	26.50 ± 2.01	$17.51 \pm 2.37^{***}$	23.33 ± 3.45
1, %	27.54 ± 2.03	29.96 ± 2.86	$61.33 \pm 3.98^{* **}$
2 and more, %	45.96 ± 2.27	52.53 ± 3.11	$15.33 \pm 2.94^{* **}$
– artificial abortions, %	15.73 ± 1.66	14.79 ± 2.21	$6.67 \pm 2.04^{* **}$
– miscarriages, %	10.14 ± 1.37	12.84 ± 2.09	$6.67 \pm 2.04^{**}$
– missed miscarriages, %	2.72 ± 1.02	5.38 ± 1.03	$0.67 \pm 0.66^{**}$
Menarche, years:	13.47 ± 0.06	13.59 ± 0.05	$13.39 \pm 0.88^{**}$
– early, %	0.83 ± 0.41	1.95 ± 0.86	1.33 ± 0.94
– late, %	9.73 ± 1.85	10.56 ± 1.40	$5.33 \pm 1.83^{**}$
Menopause, %	2.00 ± 1.14	$8.49 \pm 1.27^{***}$	$1.95 \pm 0.86^{**}$
Menstrual function disturbances, %:	64.39 ± 2.18	59.53 ± 3.06	58.00 ± 4.03
– hypermenorrhea, %	3.54 ± 1.05	$10.46 \pm 2.47^{***}$	–
– oligomenorrhea, %	2.57 ± 0.90	3.27 ± 1.44	3.45 ± 1.96
– dysmenorrhea (painful menstruation), %	61.41 ± 2.76	57.52 ± 4.00	60.92 ± 5.23
– opsomenorrhea, %	3.22 ± 1.00	5.23 ± 1.80	5.75 ± 2.50
– proyomenorrhea, %	3.45 ± 1.96	$9.80 \pm 2.40^{***}$	$2.25 \pm 0.84^{**}$
– irregular menstrual cycle, %	26.44 ± 4.73	27.01 ± 2.52	$15.33 \pm 2.94^{**}$

Note: * – $p < 0.05$ between group 1 and control group; ** – $p < 0.05$ between group 2 and control group; *** – $p < 0.05$ between group 1 and group 2

The compromised gynecological history was detected in more than a third of patients with the firstly diagnosed CE (33.13 ± 2.14 %) and recurrent CE (38.13 ± 3.03 %).

Analyzing the structure of gynecological diseases history presented in **Table 2**, it can be noted that the proportion of adenomyosis was significantly ($p < 0.05$) higher in patients with firstly diagnosed CE (9.32 ± 1.32 %) and recurrent CE (8.56 ± 1.75 %) in comparison to the control group (2.00 ± 1.14 %). Patients of group 2 were more likely to have abnormal uterine bleeding (AUB) (31.52 ± 2.90 %), Bartolin's cyst (5.84 ± 1.46 %) in history in comparison to group 1 (13.46 ± 1.55 % and 0.41 ± 0.29 % respectively), while this pathology in women of the control group was not diagnosed. Also, proportion of ovarian cysts (14.40 ± 2.19 %) and uterine fibroids (20.62 ± 2.52 %) was significantly ($p < 0.05$) higher in patients with recurrent CE in comparison to group 1 and control group.

In patients with recurrent CE a third of gynecologic diseases in the history (32.30 ± 2.92 %) and 23.60 ± 1.93 % – in patients with the firstly diagnosed CE were pelvic inflammatory diseases – chronic salpingitis, oophoritis and endometritis, significantly ($p < 0.05$) higher in comparison to the control group (3.33 ± 1.47 %).

It should be noted, that 12.22 ± 1.49 % of patients in group 1 and 21.40 ± 2.56 % of patients in group 2 had the history of laparoscopic or laparotomy surgery on internal reproductive organs, which is significantly higher ($p < 0.05$) compared to the control group, where this indicator was 6.00 ± 1.94 %.

Table 2

The structure of gynecological diseases in the history of patients with CE and women of the control group

No.	Disease	Group 1 (n=483)	Group 2 (n=257)	Control group (n=150)
1	Adenomatosis, %	1.04 ± 0.46	0.39 ± 0.39	–
2	Adenomyosis, %	9.32 ± 1.32	8.56 ± 1.75	$2.00 \pm 1.14^* \text{ **}$
3	AUB, %	13.46 ± 1.55	$31.52 \pm 2.90^{***}$	–
4	Bartolin's cyst, %	0.41 ± 0.29	$5.84 \pm 1.46^{***}$	–
5	Cervical polyp, %	4.76 ± 0.97	7.00 ± 1.59	–
6	Chronic endometritis, %	3.93 ± 0.88	5.84 ± 1.46	–
7	Chronic salpingitis, oophoritis, %	19.67 ± 1.81	$26.46 \pm 2.75^{***}$	$3.33 \pm 1.47^* \text{ **}$
8	CIN I, %	12.42 ± 1.50	11.28 ± 1.97	–
9	CIN II, %	1.86 ± 0.62	5.84 ± 1.46	–
10	CIN III, %	0.62 ± 0.36	0.78 ± 0.55	–
11	Ectopic pregnancy, %	2.90 ± 0.76	1.56 ± 0.77	–
12	Endometrial hyperplasia, %	14.29 ± 1.59	12.45 ± 2.06	–
13	Endometriosis, %	2.90 ± 0.76	1.56 ± 0.77	–
14	Ovarian apoplexy, %	3.73 ± 0.86	1.95 ± 3.33	3.33 ± 1.47
15	Ovarian cyst, %	9.11 ± 1.31	$14.40 \pm 2.19^{***}$	$6.00 \pm 1.94^{**}$
16	PCOS, %	2.28 ± 0.68	0.78 ± 0.55	1.33 ± 0.94
17	Pelvic organ prolapse, %	–	8.17 ± 1.71	–
18	Primary sterility, %	1.24 ± 0.50	1.17 ± 0.67	4.00 ± 1.60
19	Secondary sterility, %	0.62 ± 0.36	–	–
20	Uterine fibroids, %	11.39 ± 1.45	$20.62 \pm 2.52^{***}$	$3.33 \pm 1.47^* \text{ **}$

Note: * – $p < 0.05$ between group 1 and control group; ** – $p < 0.05$ between group 2 and control group; *** – $p < 0.05$ between group 1 and group 2

It was noted, that 48.25 ± 2.27 % of patients in group 1 and 47.84 ± 3.13 % – in group 2 had in the history frequent infectious diseases, this indicator was significantly ($p < 0.05$) higher in comparison to the control group (24.67 ± 3.52 %). In patients with recurrent CE the share of chronic diseases of different etiology made up 8.24 ± 1.72 %, while in patients with firstly diagnosed CE – 4.33 ± 0.92 %, in women of the control group – 1.33 ± 0.94 %. The compromised allergic history was detected in 7.00 ± 1.59 % of the patients in group 2, this indicator was significantly ($p < 0.05$) higher, than the one in the control group (2.67 ± 1.32 %). Among other diseases, the proportion of anemia should be noted, this indicator in group 1 (42.06 ± 2.24 %) and group 2 (41.96 ± 3.09 %) was almost two times higher ($p < 0.05$) in comparison to the control group (25.33 ± 3.55 %).

Firstly diagnosed CE was more frequently (in comparison to recurrent CE) ($p < 0.001$) characterized by asymptomatic course, whereas about half of patients with recurrent CE (46.69 ± 3.11 %) presented with different complaints, the most common ($p < 0.05$) – of the blood mixed discharge presence (compared with patients of group 1). Also, more often ($p < 0.001$), than patients in group 1, this category of patients was disturbed by poscoital bleeding (5.45 ± 1.42 %).

It is characteristic, that the interval from the moment of complicated symptomatic CE diagnosis to obtaining appropriate treatment in patients with recurrent CE was significantly ($p < 0.001$) longer (compared to patients of group 1) and averaged 4.05 ± 0.49 years. At the same time, it was noted, that in patients of group 2 carriage of high-risk HPV was diagnosed more often ($p < 0.05$).

The colposcopy results in the two groups of patients were not significantly different, however, in patients of group 2, the presence of non-uniform iodine uptake zones was significantly ($p < 0.05$) higher (**Table 3**).

Table 3

Features of the firstly diagnosed and recurrent CE clinical course

Indicator	Group 1 (n=483)	Group 2 (n=257)
Complaints, %:	28.57 ± 2.06	$46.69 \pm 3.11^{**}$
– lower abdominal pain, %	6.21 ± 1.10	9.34 ± 1.82
– blood mixed discharge, %	19.67 ± 1.81	$36.58 \pm 3.00^{**}$
– postcoital bleeding, %	2.07 ± 0.65	$5.45 \pm 1.42^*$
– foul smelling vaginal discharge, vaginal itching, %	7.45 ± 1.20	8.95 ± 1.78
Duration of CE presence, years	1.06 ± 0.12	$4.05 \pm 0.49^{**}$
High-risk HPV carriage, %	4.97 ± 0.99	$10.89 \pm 1.94^*$
CE diameter, cm	2.02 ± 0.08	$2.55 \pm 0.12^{**}$
Colposcopic findings:		
– acetic-white epithelium, %	12.28 ± 4.35	18.33 ± 5.00
– atypical TZ, %	–	1.67 ± 1.65
– cervical hypertrophy, %	12.28 ± 4.35	3.33 ± 2.32
– dilatation of the superficial vessels, %	12.28 ± 4.35	10.00 ± 3.87
– excretory ducts of glands, %	7.02 ± 3.38	1.67 ± 1.65
– mosaic, %	10.53 ± 4.06	13.33 ± 4.39
– non-uniform iodine uptake zone, %	3.51 ± 2.44	$15.00 \pm 4.61^*$
– Nabothian cyst, %	14.04 ± 4.60	6.67 ± 3.22
– punctuation, %	8.77 ± 3.75	11.67 ± 4.14
– TZ type 1, %	14.04 ± 4.60	18.33 ± 5.00

Note: * – $p < 0.05$ between group 1 and control group; ** – $p < 0.05$ between group 2 and control group

The highest percentage of cytological norm was found in women of the control group – 60.00 ± 4.00 % (Pap smear) and 93.33 ± 2.04 % respectively (Bethesda, 2001), which is significantly ($p < 0.05$) higher, than in patients of group 1 and 2. Identical trends were also observed in the case of type IIA and IIB (Pap smear) and ASC-US (Bethesda, 2001).

The level of leukocytes found during bacterioscopy was significantly ($p < 0.05$) higher in patients of group 1 (35.04 ± 1.73 %) and group 2 (37.75 ± 1.38 %) in comparison to the control group (13.99 ± 1.21 %) (**Table 4**).

Table 4

Features of cytology, bacterioscopy and bacteriology in patients with CE and women of control group

Indicator	Group 1 (n=483)	Group 2 (n=257)	Control group (n=150)
Cytology:			
– Pap smear			
type I, %	19.05 ± 1.79	16.34 ± 2.31	$60.00 \pm 4.00^* **$
type IIA, %	47.41 ± 2.27	48.64 ± 3.12	$33.33 \pm 3.85^* **$
type IIB, %	20.08 ± 1.82	23.35 ± 2.64	$6.67 \pm 2.04^* **$
type IIIA, %	11.80 ± 1.47	10.12 ± 1.88	–
type IIIB, %	1.66 ± 0.58	1.56 ± 0.77	–
– Bethesda (2001)			
NILM, %	66.46 ± 2.15	64.98 ± 2.98	$93.33 \pm 2.04^* **$
ASC-US, %	20.08 ± 1.82	23.35 ± 2.64	$6.67 \pm 2.04^* **$
LSIL, %	11.80 ± 1.47	10.12 ± 1.88	–
HSIL, %	1.66 ± 0.58	1.56 ± 0.77	–
Bacterioscopy:			
– flora			
bacilli, %	67.70 ± 2.13	66.15 ± 2.95	71.33 ± 3.69
cocci, %	8.70 ± 1.28	8.56 ± 1.75	4.67 ± 1.72
mixed, %	23.60 ± 1.93	25.29 ± 2.71	24.00 ± 3.49
– leucocytes	35.04 ± 1.73	37.75 ± 1.38	$13.99 \pm 1.21^* **$
Bacteriology:			
– C. albicans, %	19.07 ± 2.45	26.29 ± 2.00	$13.33 \pm 2.78^* **$
– G. vaginalis, %	8.00 ± 2.22	7.00 ± 1.59	5.80 ± 1.06
– Tr. vaginalis, %	2.33 ± 0.94	3.31 ± 0.81	$0.67 \pm 0.66^{**}$
– pseudomycosis, %	2.72 ± 1.02	4.55 ± 0.95	2.67 ± 1.32

Note: * – $p < 0.05$ between group 1 and control group; ** – $p < 0.05$ between group 2 and control group

Conservative treatment was taken by patients with firstly diagnosed CE significantly ($p < 0.001$) more rarely (26.29 ± 2.00 % of cases) and mainly included local NSAIDs (28.57 ± 2.06 %) and wide spectrum antibiotic therapy (20.29 ± 1.83 %). Only in 11.39 ± 1.45 % of cases vaginal antiseptics were used, in 3.93 ± 0.88 % of cases- treatment aimed to restore the hormonal balance, in 1.86 ± 0.62 % of cases – drugs that normalize the state of vaginal microbiota. Drugs that affect the reparation and regeneration of the epithelium have not been prescribed in any case. A similar trend was observed in patients with recurrent CE.

While analyzing the structure of surgical treatment methods, it was noted, that patients with recurrent CE significantly ($p<0.05$) more often underwent diathermoconization ($24.12\pm 2.67\%$) and cryodestruction of the cervix ($8.17\pm 1.71\%$) in comparison to patients of group 1. The treatment method most often performed in patients of both groups was particular diathermoconization (**Fig. 1**).

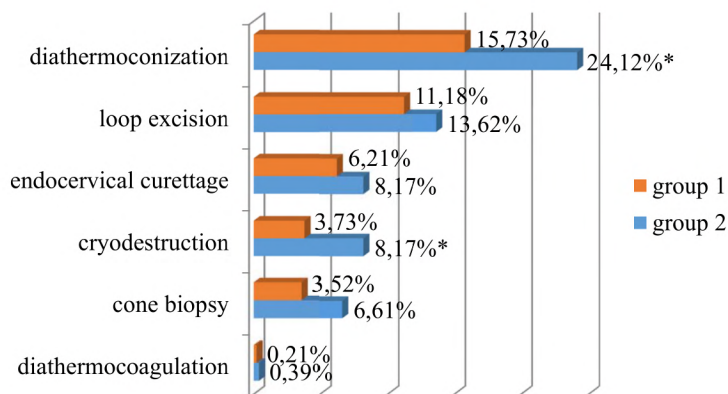


Fig. 1. The structure of CE surgical treatment methods (* – $p<0.05$ between group 1 and group 2)

It should be noted, that $5.80\pm 1.06\%$ of group 1 patients and $6.23\pm 1.51\%$ of group 2 patients, who underwent either loop excision, or diathermoconization at the time of the intervention were nulliparous.

5. Discussion

In recent years the medical community attitude to the CE has shifted its vector: with the introduction of colposcopy and the implementation of the Bethesda system (2001) principles [17] in the routine medical practice, CE is now commonly interpreted as a physiological state, which does not require any intervention.

However, there is a problem unsolved concerning patients category, in which the presence of CE is associated with definite clinical symptoms, which create discomfort and may affect the quality of life, as well as the pathological CE, when the epithelization is complicated or impeded due to the presence of hormonal, local immunity, vaginal biocenosis disorders, HPV infection and chronic vaginal or cervical inflammatory processes.

We studied the clinical course and recurrence features of both firstly diagnosed and recurrent CE, which should be taken into account when choosing the complicated CE optimal treatment tactics, also aiming to prevent its relapse in the future.

Analyzing the obstetrical and gynecological history (**Table 1**), it was found that patients with recurrent CE had significantly ($p<0.05$) more pregnancies, than the ones with firstly diagnosed CE and healthy women. A similar connection was noted in other studies [18]. The proportion of women with high parity (2 and more labors per vias naturalis) in group 1 and group 2 was three times higher ($p<0.05$), than in group 3, which can be explained by the probable relationship between obstetric trauma and CE in this contingent of patients [19].

The proportion of artificial abortions in the history of patients in groups 1 and 2 was more than two times higher ($p<0.05$), than in women of the control group. In addition, group 2 patients had significantly more ($p<0.05$) miscarriages and missed miscarriages in comparison to the control group.

It was also found that hypermenorrhea was significantly ($p<0.05$) more commonly diagnosed in patients of group 2 in comparison to group 1, this disturbance was not found among women of control group. In group 2, the proportion of women with polymenorrhea was three times higher ($p<0.05$), than in group 1 and control group respectively.

Compromised gynecological history (especially concerning the pathology related with hormonal disturbances and chronic pelvic inflammatory diseases) was more typical for patients with CE: this indicator was significantly ($p<0.05$) higher in group 1 and group 2, than in the control group.

Almost half of patients in group 1 and group 2 had in the history frequent (more than 3 times a year) infectious diseases (ARI, influenza, pneumonia), this indicator was significantly ($p<0.05$) higher in comparison to the control group. In patients with recurrent CE chronic diseases of different etiology (in particular, chronic bronchitis, tonsillitis, pyelonephritis) were diagnosed more often ($p<0.05$), than in group 1 and control group.

The size of the CE, measured during colposcopy, in patients of group 2, compared to patients of group 1, was significantly ($p<0.001$) greater. It was found, that in the presence of CE with a diameter greater than 3.5 mm, the risk of postcoital bleeding increases [20], which explains the higher prevalence of this symptom in patients with recurrent CE.

The frequent presence of cervicitis cytological indicators in patients with firstly diagnosed and recurrent CE correlates with the results of other studies: Singh et al. [21], studying the etiology of inflammatory cervical smears, found CE in a significant proportion of patients; moreover, the presence of CE can be associated with recurrent cervicitis [19].

This fact should be taken into account when choosing the treatment method for complicated CE, as it has been established that, in particular, cryodestruction of CE in patients with ≥ 3 episodes of cervicitis exacerbation during the past 6 months was 9 times less effective [19].

Vaginal candidiasis was diagnosed more often ($p<0.05$) in patients with firstly diagnosed and recurrent CE in comparison with control group (**Table 4**). A similar feature of vaginal microbiota was discovered by other researchers [22], who suggest that the dominance of *C. albicans* is a favorable factor for CE existence [23].

As our study showed, in practice, surgical treatment methods of complicated CE are still commonly used, unlike the treatment aimed at normalizing hormonal balance, vaginal microbiota, and promoting the processes of cervical epithelium regeneration. The shifting of the emphasis from the causes of CE and its long-term existence to the elimination of the consequences - the CE itself, is probably also one of the recurrence causes.

6. Conclusions

1. Complicated recurrent CE should not be treated as an isolated pathology, that can be eliminated radically and quickly using only local means (both drugs and surgery), but as a possible indicator of reproductive health in women and should not be neglected.

2. The risk factors of recurrent CE include: 2 or more labors per vias naturalis, abortions, artificial abortions, miscarriages and missed miscarriages in the history, late menarche, menstrual function disorders such as polymenorrhea, compromised gynecological history (the diseases provoked by hormonal homeostasis disorders: adenomyosis, ovarian cysts, uterine fibroids, as well as chronic pelvic inflammatory diseases), the presence of extragenital pathology (frequent infectious diseases, chronic diseases, compromised allergological history, anemia).

3. Recurrent CE is characterized by symptomatic clinical course, it is more often combined with the HPV carriage, colpitis and cervicitis (often of mixed etiology), disturbances of vaginal microbiota, but at the same time, patients tend to postpone treatment for a long time.

4. Among the used first-line treatment methods of firstly diagnosed as well as recurrent CE, the lion's share takes surgical treatment in combination with the common use of wide spectrum antibacterial therapy and non-steroidal anti-inflammatory drugs.

5. Thus, recurrent symptomatic complicated CE should be interpreted as a complex problem that requires a multi-polar approach aimed at hormonal homeostasis, local immunity, and vaginal biocenosis normalizing. These measures should precede invasive treatment and further create optimal conditions for the cervix regeneration.

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PROGNOSTIC VALUE OF IMMUNOHISTOCHEMICAL MARKERS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is a potentially curable disease, but standard treatment is not effective enough for all patients. That is why so important to identify high risk patients who need more aggressive therapy at the time of diagnosis. Nowadays prognosis for patients with DLBCL is based on International prognostic index (IPI). However, this index consists of only clinical parameters and does not include the biological characteristics of the tumour. Immunohistochemistry (IHC) markers could also play a prognostic role. There are some publications regarding predictive and prognostic role of expression of Bcl-2, Bcl-6, MUM1, CD10 and CD30, but their results are controversial. The aim of our study was to analyze prognostic role of these markers, to compare survival of patients with positive and negative expression of these markers and to build a prognostic model which include biological parameters for identifying high risk patients. There were statistically significant differences in EFS between the group of patients with negative and positive expression of CD10 (51.5 % versus 72.5 %, $p=0.01$) and in OS between the group of patients with negative and positive expression of Bcl-6 (61.1 % versus 79.6 %, $p=0.03$). Six-factors nonlinear neural network prediction model (MLP_6) was built. The sensitivity of the model is 63.2 % (95 % CI 49.3 % – 75.6 %), specificity – 85.2 % (95 % CI 79.1 % – 90.1 %). Prognostic factors include negative IHC expression of Bcl-6, CD10, non-GCB molecular subtype (according to algorithm Hans), gender (male), advanced Ann-Arbor stages, >2 extranodal involvement. Our nonlinear neural network prediction model could improve prognostic role of IPI by adding of biological tumour characteristics (IHC expression of CD10, Bcl-6, molecular subtype by IHC algorithm).

Keywords: diffuse large B-cell lymphoma, prognosis, predictive model, biological characteristics.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is a potentially curable disease, but unfortunately approximately 40 % of patients are refractory to the primary therapy or develop relapse after first line therapy [1, 2].

Standard of treatment is not effective enough for this group of patients. That is why so important to identify high risk patients who need more aggressive therapy at the time of diagnosis. On the other hand there are a lot of new drugs recently and searching for prognostic markers is extremely important for risk stratification of patients in order to individualize treatment.

Nowadays prognosis for patients with DLBCL is based on International prognostic index (IPI) [3]. IPI was described in 1993 based on the analysis of 3273 patients with aggressive non-Hodgkin lymphoma in 6 Oncologic centers, received CHOP-like treatment. Based on such negative prognostic factors as age more than 60 years old, elevated lactate dehydrogenase, status ECOG >1, III–IV stages, extranodal sites of involvement >1, patients were divided into four risk groups: low (0–1 factor), low-intermediate (2 factors), high-intermediate (3 factors) and high risk (4–5 factors) with 5-year overall survival (OS) 73 %, 51 %, 43 % i 26 % accordingly [3]. However, despite its wide spread use in clinical practice and predictive value, this index consists of only clinical parameters and does not include the biological characteristics of the tumour. Immunohistochemistry (IHC) markers which are used for diagnosis of lymphoma and play important role in its pathogenesis could also play a prognostic role. There are some publications regarding predictive and prognostic role of expression of Bcl-2, Bcl-6, MUM1, CD10 and CD30, but their results are controversial.

Positive expression of MUM1 is associated with inferior outcome according to the several study results [4, 5]. And some study results demonstrated positive prognosis in case of positive expression of this marker [6, 7].

According to the results of Berglund and Sjöet al positive expression of CD10 is a positive prognostic factor [8, 9] and have no prognostic impact according other results [10].

Negative prognostic influence of Bcl-2 was demonstrated in several studies [8, 11]. And according to other results there is no prognostic value of this marker [12, 13]. Negative Bcl-6 expression is associated with inferior survival according Horn and his colleagues [14, 15] and there is no prognostic value of this marker according others results [16].

2. Aim of research

The aim of the study was to identify the prognostic role of immunohistochemical markers and to find prognostic model for detecting patients with survival less than 12 months.

3. Materials and methods

In the retrospective analysis were included 349 patients with newly diagnosed DLBCL who were treated at the National Cancer Institute (Kyiv, Ukraine) from December, 2014 to December, 2017. IHC expression of Bcl-2, Bcl-6, MUM1, C-MYC, CD10 or CD30 in these patients were compared.

IPI factors and survival between groups of patients with positive and negative expression of IHC markers were compared. For identifying of molecular subtype (germinal center (GCB) DLBCL or non-germinal center DLBCL (non-GCB)) immunohistochemical Hans algorithm was used [17].

Group comparisons were performed by means of χ^2 and Student's *t* tests. For time to event analyses we used EZR v. 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan, 2017), applying Kaplan-Meier survival estimates with the end-point of overall survival, defined as the time from initial diagnosis to death from any cause [18]. P values less than 0.05 were considered statistically significant.

For the analysis of the risk of death in the first 12 months after diagnosis and construction of the system for predicting this risk, the method of analysis of neural network models [19] was used. To select a minimum set of parameters related to the risk of early death, the method of genetic algorithm for selecting variables [19] was used.

In order to assess the adequacy of the forecasting model, the method of constructing and analyzing the operational characteristics curves of the models (ROC curves) was used, the estimation

of the quality of the prediction was made by assessing of area under the operational characteristics curve (AUC), sensitivity, specificity, predictability of the positive result, and the prediction of the negative outcome, the corresponding 95 % of the indicators were calculated [20].

4. Result

There were 162 (46.4 %) men and 187 (53.6 %) women. Age of patients was from 17 to 86 years, median age – 54.0 ± 1.1 years of old (95 % CI 50-56). Early stages (I–II) were registered in 182 (52.1 %) of patients, advanced stages (III–IV) – in 167 (47.9 %). There were B-symptoms in 127 (36.4 %) of patients. There was low and low-intermediate IPI in 238 (68.2 %) of patients and 111 (31.8 %) high and high-intermediate risk patients. There were 132 (37.8 %) patients older than 60 years old, 153 (43.8 %) patients with elevated LDH, 167 (47.9 %) patients with advanced stages, 89 (25.5 %) patients with ECOG >2 and 99 (28.4 %) patients with more than two extranodal sites of involvement. Rituximab-based chemotherapy received 186 of patients (53.3 %), patients received 1-8 courses of chemotherapy (6 ± 0.1 courses, 95 % CI 5.0–7.0). Radiation therapy received 116 of patients (33.2 %). Median follow up was 12.7 months.

Positive expression of Bcl-2, Bcl-6, MUM1, C-MYC, CD10 or CD30 was registered in 79.4 %, 56.8 %, 63.7 %, 28 %, 38.2 %, and 23.5 % accordingly (**Fig. 1**).

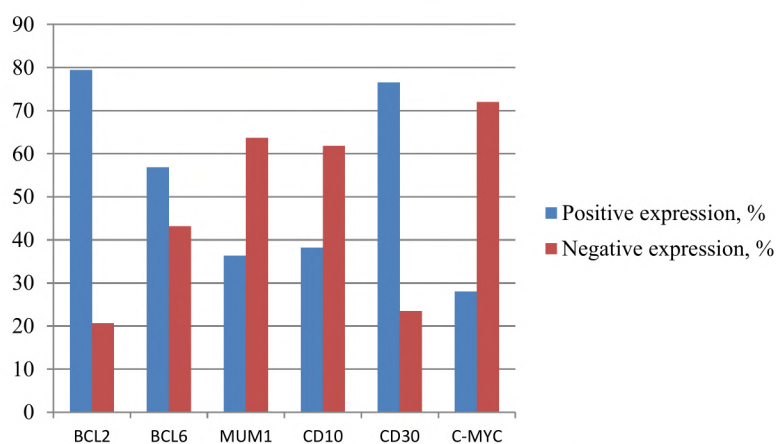


Fig. 1. Incidence of positive (blue color) and negative (red color) expression of IHC markers

In the **Table 1** IPI factors distribution depending on positive or negative expression of IHC markers is demonstrated.

Table 1

IPI risk factors in the group with positive and negative expression of IHC markers

% IHC	Age>60	Stage III-IV	>2 extranodal sites	LDN>N	ECOG>2	IPI 3-5
1	2	3	4	5	6	7
Bcl-2+	42	53.5	32	55.3	28	38
Bcl-2-	28.8	35	23	63.6	23	21
p	0.12	0.08	0,27	0.41	0.69	0.04
Bcl-6+	33.3	45.8	31,9	59	25.4	34.2
Bcl-6-	39.5	56	32,9	63,4	31.8	35.1
p	0.43	0,18	0.99	0.68	0.38	0.99
MUM1+	41.5	44.5	24.4	55.8	24.4	32.3
MUM1-	35.1	44.5	31.1	52.2	22.9	28.3

Continuation of Table 1

1	2	3	4	5	6	7
p	0.45	0.7	0.67	0.76	0.95	1.0
CD10+	30.9	38	27.4	51.3	22.6	27.4
CD10-	42.6	51.4	30.4	55	25	33.8
p	0.11	0.053	0.75	0.74	0.82	0.39
CD30+	25.9	44.4	33.3	54.5	22.2	18.5
CD30-	38.6	54.4	26.4	50	27.7	31.8
p	0.32	0.48	0.97	0.89	0.91	0.27
C-MYC +	37.1	57.1	37.1	51.5	40	42.8
C-MYC –	44.4	54.4	38.2	72.7	32.5	50
p	0.59	0.08	0.95	0.053	0.57	0.6

There were no difference in age > 60 years old, advanced stages, elevated LDH, more than two extranodal involvements, ECOG > 2 between patients with positive and negative expression of Bcl-2, Bcl-6, CD10, CD30, MUM1 та C-MYC. There was similar distribution of high risk patient between with positive and negative expression of Bcl-6, CD10, CD30, MUM1 та C-MYC. There were more high risk patients according IPI in the group of patients with positive Bcl-2 expression (p=0.04).

Two-year overall survival (OS) and event-free survival (EFS) were analyzed and compared between group of patients with positive and negative expression of IHC markers (**Table 2**).

Table 2

Two-year overall and event-free survival in patients with positive and negative expression of IHC markers

IHC, %	EFS, %	95 % CI	OS, %	95 % CI
Bcl-2+	65.5±7.35	49.1–77.8	73.23.8±	64.8–79.8
Bcl-2–	61.44.1±	52.9–68.9	76.36.7±	59.9–86.6
p	0.6			0.8
Bcl-6+	52.96.36±	39.8–64.4	79.64.3±	69.5–86.6
Bcl-6–	63.25.17±	52.2–72.4	61.16.3±	47.6–72.1
p	0.2			0.03
MUM1+	63.04.86±	52.7–71.7	77.04.9±	65.5–85.1
MUM1–	57.07.21±	41.8–69.7	66.76.8±	51.5–78.1
p	0.7			0.1
CD10+	72.55.58±	59.8–81.7	77.05.8±	62.9–86.3
CD10–	51.55.38±	42.7–63.0	68.35±	57.1–77.1
p	0.01			0.2
CD30+	53.111.1±	29.9–71.8	65.410.9±	39.9–82.2
CD30-	54.66.6±	40.8–66.4	68.66.4±	54.1–79.3
p	0.9		0.805	
MYC+	47.912.85±	22.2–69.7	67.514.3±	32.2–87.3
MYC–	59.46.03±	46.6–70.0	69.06.15±	55.2–79.3
p	0.9		0.5	

In the group of patients with negative expression of CD10 there EFS was worse comparing to the group of patients with positive expression of CD10 (51.5 % versus 72.5 %, $p=0.01$). In the group of patients with negative expression of Bcl-6 there was worse OS comparing to the group of patients with positive expression of Bcl-6 (61.1 % versus 79.6 %, $p=0.03$) (**Fig. 2**).

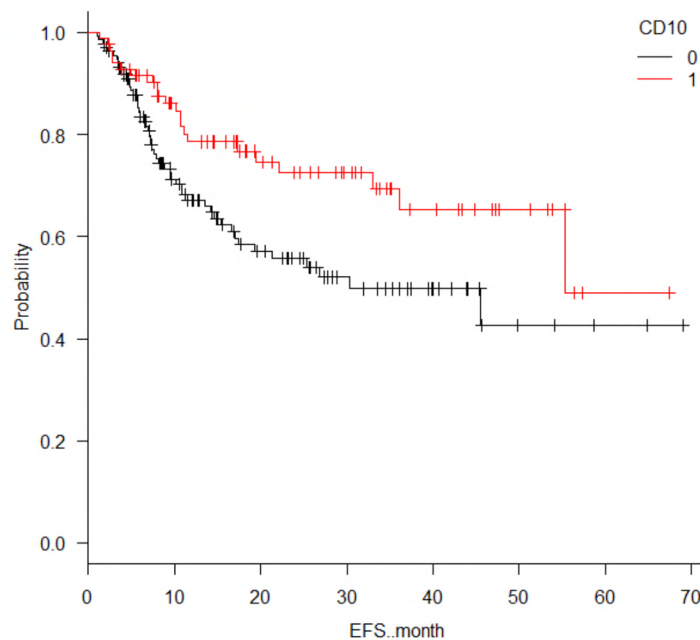
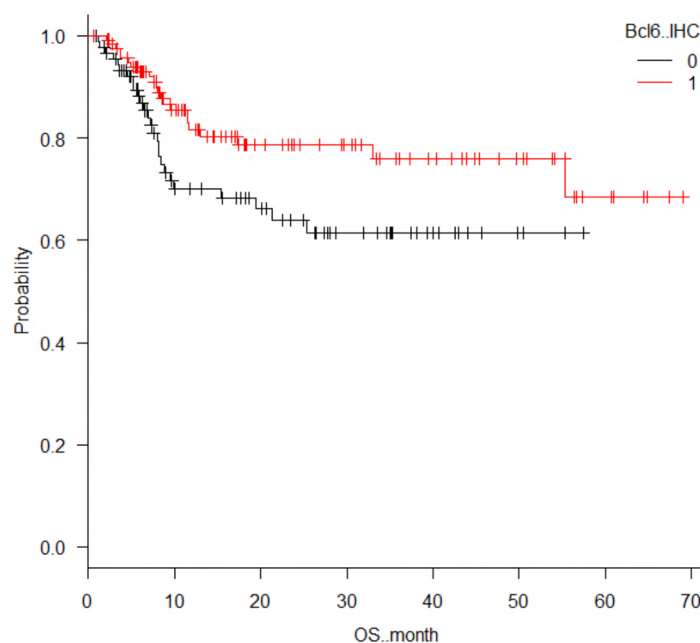
*a**b*

Fig. 2. Survival of DLBCL patients depending on IHC markers expression:
a – EFS of patients with positive (1, red line) and negative (0, black line) expression of CD10;
b – OS of patients with positive (1, red line) and negative (0, black line) expression of Bcl-6

An analysis of the association of treatment results of patients with DLBCL with different factor characteristics (27 clinical characteristics of patients, including IPI factors, IHC expression

of Bcl-2, MUM1, Bcl-6, CD10, CD30, C-MYC etc.) was performed. As a starting point, the likelihood of survival for more than 12 months was chosen. Outcome variable $Y=0$ for patients with $OS>12$ months (176 patients), $Y=1$ for patients with $OS \leq 12$ months (57 patients).

The 27-factor linear neural network model for prediction survival less than 12 month was built with 79.3 % (95 % IU 58.5-83.0 %) of sensitivity and 80.1 % (95 % IU 73.4 % – 85.7 %) of specificity.

As a result of selection of the minimum number of the most significant factors the method of genetic selection algorithm was used [1]. There were six characteristics for prognosing survival <12 months: negative IHC expression of Bcl-6 (X1), CD10 (X2), non-GCB molecular subtype (according to algorithm Hans) (X3), gender (male) (X4), advanced Ann-Arbor stages (X5), >2 extranodal involvement (X6). Based on this characteristics 6-factor linear prediction model (Lin_6) with 86.0 % (95 % CI 74.2 % – 93.7 %) of sensitivity and 62.5 % (95 % CI 54.9 % – 69.7 %) of specificity was built.

In order to find out the possible nonlinear relationships of the distinguished factor characteristics and the risk of low survival, in the third stage of the analysis, a six-factors nonlinear neural network prediction model (such as a multi-layered perceptron with one hidden layer) (MLP_6) with the same parameters was built. The sensitivity of the model is 63.2 % (95 % CI 49.3 % – 75.6 %), specificity – 85.2 % (95 % CI 79.1 % – 90.1 %). **Fig. 3** shows the architecture of this model.

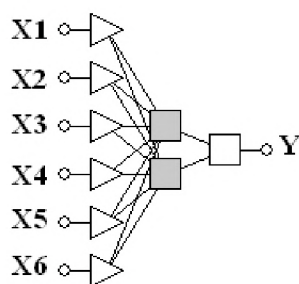


Fig. 3. Architecture of six-factor nonlinear neural network prediction model for prognosis of low survival. White triangles are the neurons of the input layer, the gray squares - the hidden layer neurons, the white square - the neuron of the initial layer of the network

The method of comparison of curves of operational characteristics of models was used for comparison of prognosis qualities of three constructed predicting models: Lin_27, Lin_6 and MLP_6 (**Fig. 4**).

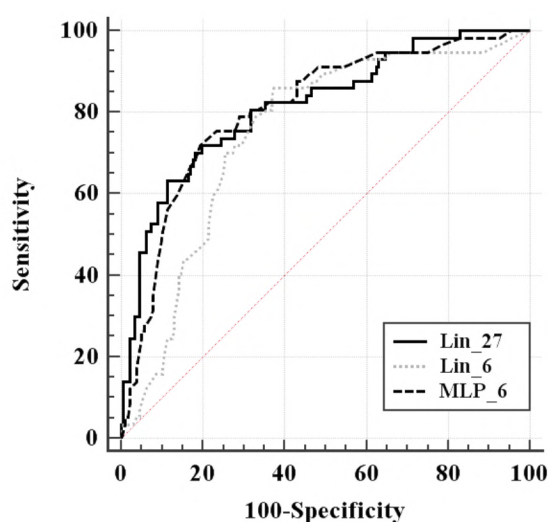


Fig. 4. Curves of operational characteristics of prognosis qualities of three constructed predicting models

According to this analysis of ROC-curves there were no differences between Lin_27 and MLP_6.

To choose the optimal threshold, the decision was made based on minimizing the prediction error (minimizing the error of false negative + false positive results). At the same time, the same value of errors was chosen. When conducting an optimization, the threshold value $Y_{crit}=0.527$, while $Y \leq Y_{crit}$ in the model predicts the risk of low survival, otherwise the prognosis is favorable.

Group of patients with positive and negative expression of IHC markers Bcl-2, Bcl-6, CD10, CD30, MUM1 and C-MYC were comparable in such characteristics as old age, advanced stages, elevated LDH, more than two extranodal involvements, ECOG>2. There were more high risk patients according to IPI in the group of patients with positive Bcl-2 expression ($p=0.04$).

There was a statistically significant difference in EFS between the group of patients with negative and positive expression of CD10 (51.5 % versus 72.5 %, $p=0.01$) and in OS between the group of patients with negative and positive expression of Bcl-6 (61.1 % versus 79.6 %, $p=0.03$).

Six-factors nonlinear neural network prediction model (MLP_6) was built. The sensitivity of the model is 63.2 % (95 % CI 49.3 % – 75.6 %), specificity – 85.2 % (95 % CI 79.1 % – 90.1 %). Prognostic factors include negative IHC expression of Bcl-6, CD10, non-GCB molecular subtype (according to algorithm Hans), gender (male), advanced Ann-Arbor stages, >2 extranodal involvement.

5. Discussion

Despite more numbers of high risk patients according to IPI were in the group of patients with positive Bcl-2 expression, expression of this IHC marker did not have any influence on survival and did not show significant value during multivariable analysis. The results of study by Wilson W. H. and colleagues also demonstrated the absence of prognostic role of Bcl-2 expression.

Our analysis suggests association between negative IHC expression of CD10 and Bcl-6 and inferior outcome in patients with DLBCL. According to the Hans' et al. and Berglund's et al. data, positive expression of CD10 and Bcl6 also are associated with better survival.

This suggestion was also confirmed in multivariable analysis where six independent factors of poor survival were identified. Apart from 2 IPI factors (age, extranodal involvements) 1 new clinical factor (gender) and 3 biological tumor characteristics were identified. This combination of three new biological factors in our prognostic model was not described in the literature before and is a valuable addition to the IPI for identifying poor prognosis patients with DLBCL.

6. Conclusion

1. IPI is a standard, but not perfect predictor of prognosis in patients with DLBCL and it includes only clinical patients' characteristics.
2. Our nonlinear neural network prediction model could improve prognostic role of IPI by adding of biological tumor characteristics (IHC expression of CD10, Bcl-6, molecular subtype by IHC algorithm).
3. This prognostic model needs further confirmation in larger studies.

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LEVEL OF GLYCATION END PRODUCTS AND GALACTIN-3 IN PATIENTS WITH CHRONIC HEART FAILURE AND ATRIAL FIBRILLATION IN DEPENDENCE ON THE AGE AND RENAL FUNCTIONAL

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Abstract

Aim. To analyze the changes of advanced glycation end products (AGE) and galectin-3, their relationship in patients with chronic heart failure (CHF) and atrial fibrillation (AF), depending on the renal functional and age.

Material and methods. 30 patients with CHF II-III FC according to the classification of the New York Heart Association (NYHA) with preserved systolic function were examined (EF>45 %, mean 58.73 [45.38, 88.00] %), mean age 66.86 [46.00, 85.00] years who were on treatment in the Regional Hospital after

I. I. Mechnikov. Men accounted for 60 % (18 people), women – 40 % (12 people). All patients were with AF: 17 (56.7 %) – with a permanent form, 13 (43.3 %) – with persistent. The serum creatinine level was determined and the GFR was calculated by CKD-EPI. The level of galectin-3 in the blood was determined by immunofermentive analis using the “Human Galectin-3 Platinum ELISA” kit (GmbH, Austria) on the Stat Fax 2100 (USA) immunofermentive plate analyzer. The fluorescent AGEs in plasma were analysed by quantitative autofluorescence (fluorimeter Hoefer DQ 2000, USA) with fixed spectrum of excitation at 460 nm with 20 % quinine solution as a standard with results expressed with conversion to glycated albumin.

Results. AGE (mean 1.579 [0.884, 2.796]) and galectin-3 (mean 8.542 [2.72, 17.73]) levels increased in 83.3 % (25 patients) and 23.3 % (7 patients) respectively. The level of galectin-3 increased with age (by 33.18 %, $p<0.05$), while the level of AGE did not have significant changes. Levels of AGE and galectin-3 increased with a decrease in GFR (by 18.9 %, $p<0.05$ and 18.44 %, $p<0.05$, respectively). The level of AGE and serum creatinine correlated to GFR ($r=0.25$, $p<0.05$ and $r=-0.22$, $p<0.05$, respectively), while the level of galectin-3 correlated to GFR ($r=-0.16$, $p<0.05$). Intake of irbesartan reduced the level of galectin-3 (by 21.66 %) and AGE (by 4.9 %). The level of serum creatinine was decreased (by 4.89 %) and GFR was increased (by 6.3 %) compared with the intake of ACE inhibitors (by 2.9 % and 1.02 %, respectively).

Discussion. The results showed changes in AGE with a decrease in GFR in patients with CHF and AF, which makes it possible to consider AGE, as a marker of cardiorenal syndrome and makes it promising to further study it. Galectin-3 showed itself not only as a diagnostic marker, but also as a dynamic (positive effect of using irbesartan). Irbesartan had comparable clinical efficacy with ACE inhibitors in patients with CHF and AF.

Conclusions. Among patients with CHF and AF, the level of AGE was increased 83.3 % of patients, galectin-3 – in 23.3 %. Depending on the age, priority was given to galectin-3 (an increase of 33.18 %, $p<0.05$). The levels of AGE and galectin-3 depends of GFR and were increased (by 18.9 % and 18.44 %, $p<0.05$ respectively). The use of irbesartan showed a more significant positive effect on the level of galectin-3 and AGE with GFR in the range $<60\geq 30$ ml/min/1.73 m².

Keywords: glycation end products, galectin-3, glomerular filtration rate, chronic heart failure, atrial fibrillation.

1. Introduction

Chronic cardiovascular diseases (CVD) and chronic kidney diseases (KD) are closely interrelated, and they share common risk factors. In the context of CVD and KD, interest in post-translational modifications (PTMs) of proteins has significantly increased. The analysis of PTMs can be useful in identifying mechanisms that play a role in the genesis and/or progression of CVD and KD, since proteins are constantly exposed to various plasma and tissue components under different pathophysiological conditions [1].

Glycation is the main cause of spontaneous disruption of the structure of intracellular and extracellular proteins of different physiological systems. In 0.1–0.2 % of cases, glycation passes through the residues of lysine and arginine [2]. In some areas where the metabolism of proteins is limited (for example, in the lens of the eye), the degree of glycation may increase in 10 times [2]. There are many products of the addition of glucose to proteins of tissues and body fluids in vivo. The earliest product of the addition of glucose to the protein is Nε-fructosyl-lysine (PL), with the slow degradation of which various advanced glycation end products (AGE) are formed. Dicarbonyl compounds of endogenous origin, as well as glyoxal, methylglyoxal and 3-deoxyglucosone have an expressed ability to glycate proteins. They are formed by the degradation of glycated proteins, intermediate metabolites of glycolysis and lipid peroxidation. Dicarbonyl compounds directly react with proteins to form AGE [2]. In the largest amount, hydroimidazolones are formed as the final glycation products, which are derivatives of arginine residues modified by glyoxal, methylglyoxal and 3-deoxyglucosone (3-DG), Nε-(5-hydro-4-imidazolone-2-yl) ornithine (G-H1), Nε-(5-hydro-5-methyl-4-imidazolone-2-yl)ornithine (MG-H1) and Nε-(5-hydro-5-(2,3,4-trihydroxybutyl) – 4-imidazolone-2-yl)ornithine and related structural isomers (3DG-H). Other widely studied AGEs are Nε-carboxymethyl-lysine (CML) and Nε-carboxyethyl-lysine (CEL), as well as derivatives of cross-linking proteins – pentosidine and glucosepane [2].

Initially, glycation was considered a reaction of post-translational modification of proteins, primarily extracellular ones. It was assumed, that AGE slowly accumulates in the body throughout life, and the concentrations of AGE reflect the process of accumulation of attachment products. However, this is only true for chemically stable AGEs formed from long-lived proteins, while in physiological conditions PL and some other AGE (in particular, hydroimidazolones) have a relatively short half-life (2–6 weeks) and can be formed from intracellular and short-lived extracellular proteins. In the degradation of proteins with glycation altered by structure, glycation products are released [2]. In vivo peptides of low molecular weight are found in small concentrations in the blood plasma and in urine, and they can also be glycated. The products of glycation of proteins and peptides are called “remnants of glycation products”, which corresponds to the nomenclature of amino acid residues in the composition of proteins. Free glycation products are glycated amino acids formed with complete proteolysis of glycated proteins and glycation of amino acids. They are excreted in the urine [2].

Glycation is the main cause of spontaneous breakdown of the structure of proteins in the body. AGE stimulates various pro-inflammatory and signaling metabolic pathways, and their excessive accumulation in the body is considered as one of the main factors in the pathogenesis of cardiovascular diseases [3]. The participation of AGE in the pathogenesis of vascular complications is confirmed by the following experimental data:

- 1) accumulation of AGE residues at the sites of vascular injuries;
- 2) against the background of a change in the structure of vascular cell proteins under the influence of AGE in vitro, cell dysfunction is observed;
- 3) inhibitors of AGE formation suppress the development of vascular complications (according to the data of the conducted experiments and clinical studies).

Atrial fibrillation (AF) is one of the most common arrhythmias, a prevalence that increases as the population age growth [4]. Molecular mechanisms involved in atrial remodeling are not fully understood, but it is known that the pro-fibrosis and proinflammatory mechanisms increase the risk of AF. One such mechanism that occurs with aging involves the non-enzymatic binding of proteins and lipids leading to the formation of AGE [4]. AGE can play a role in the pathophysiology of PL through their interaction with cellular receptors for AGE (RAGE), which causes inflammation

and fibrosis through successive activation of the nuclear factor kappa B and increased expression of cytokines and adhesion molecules. Recent studies have shown that the interaction of AGE and RAGE can contribute to the onset of inflammation and oxidative stress [4]. These processes may also participate in the pathogenesis of AF, but their role remains debatable.

Various proteins are considered as specific receptors for AGE-modified proteins. Among the receptors located on the cell surface, they include the receptor of phagocytes, the receptor of the advanced glycation end products (RAGE) and galectin-3. Other AGE-binding proteins are localized in the cytoplasm and, possibly, they are not receptors for AGE [2]. Galectin-3 is considered as an AGE receptor, as evidenced by the ability of high-glycated albumin to interact with recombinant galectin-3, as well as the specific capture of galectin-3 by macrophages of RAW mice by the same ligand [2]. In vitro galectin-3 provides protection for mesangial cells, prevents the attack of infiltrating macrophages and cytotoxic effects of TGF- β , affects the interaction of proteoglycans during angiogenesis, and also has other intracellular and extracellular effects in vivo [2]. Indeed, galectin-3 has a pro-inflammatory effect: in the case of nephropathy, galectin-3+ infiltrates macrophages in the distal tubules and glomerulus of the kidneys, and the nature of this infiltration correlates with the severity of nephropathy [2, 5]. Thus, proteins classified as AGE receptors are involved in the pathogenesis of vascular complications, but it is not completely known whether the functional activity of these receptors is enhanced under the influence of AGE-modified proteins in vivo [2]. It should be noted, that galectin-3 promotes the migration of macrophages, the proliferation of fibroblasts and the synthesis of collagen in cardiomyocytes [6, 7]. Increased expression of galectin-3 causes remodeling of the left ventricle (LV), which is accompanied by cardiac dysfunction and a decrease in the contractile function of the heart [8, 9].

It is known that the accumulation of AGE is one of the causes of CHF, nephropathy. Studies have shown that *angiotensin II receptor blockers* (ARBs) can reduce the ability to bind AGE to hypertensive patients. In this regard, our interest is the fact of studying glycation products in patients with CHF and AF, depending on the renal functional.

2. Aim of the research

To analyze the changes of advanced glycation end products (AGE) and galectin-3, their relationship in patients with chronic heart failure (CHF) and atrial fibrillation (AF), depending on the renal functional and age.

3. Materials and methods

30 patients with CHF II-III FC according to the classification of the New York Heart Association (NYHA) with preserved systolic function were examined (EF>45 %, mean 58.73 [45.38, 88.00] %), mean age 66.86 [46.00, 85.00] years who were on treatment in the Regional Hospital after I. I. Mechnikov. The study was conducted from December 2016 to December 2017. The observation period was 3 months. Among patients were 60 % (18 people) men and 40 % (12 people) women. All patients were with AF: 17 (56.7 %) – with a permanent form, 13 (43.3 %) – with persistent.

Verification of the diagnosis of CHF and AF was conducted according to the recommendations of the European Society of Cardiology [10, 11].

Inclusion criteria were the presence of CHF with a preserved ejection fraction (EF>45 %) under conditions of stably selected drug therapy, AF, glomerular filtration rate (GFR) <90 \geq 30 ml/min/1.73 m² and informed consent of the patient.

Exclusion criteria were patients with CHF IV FC and acute heart failure; acute coronary syndrome; myocardial infarction and stroke up to 6 months; significant valvular heart disease, GFR<30 ml/min/1.73 m².

For clinical evaluation of renal function, serum creatinine was determined using colorimetric methods and the GFR was calculated by CKD-EPI according to the recommendations of the American National Federation of the Kidneys [12].

All patients were assessed with galectin-3 blood levels by immunofermentive analysis using the “Human Galectin-3 Platinum ELISA” kit (GmbH, Austria) on the Stat Fax 2100 (USA) immunoassay plate analyzer. The base level was taken as 0 ng/ml.

The fluorescent AGEs in plasma were analysed by quantitative autofluorescence (fluorimeter Hoefer DQ 2000, USA) with fixed spectrum of excitation at 460 nm with 20 % quinine solution as a standard with results expressed with conversion to glycated albumin.

15 patients (the main group) who prior to inclusion in the study did not received ARBs or had a poor tolerance of ACE inhibitors, irbesartan was used at a dose of 150 mg orally once a day, with an insufficient antihypertensive effect (BP>140/90 mm Hg) after 1 week the dose of the drug was increased to 300 mg per day. 15 patients (comparison group) continued to receive therapy with ACE inhibitors.

For statistical analysis of data was used STATISTICA v.6.1 (Statsoft Inc., USA) license program. Categorical variables were expressed as frequencies and percentages. With regard to continuous variables, the assumption of normality was tested with the Kolmogorov-Smirnov test. The variables that follow a normal distribution were expressed as mean \pm standard deviation. The remaining variables were expressed as median and interquartile range. [13].

4. Results

During the examination, arterial hypertension (AH) of stage II was registered in 19 (63.3 %), stage III – in 5 (16.6 %) patients; AH of I degree – in 3 (10 %), II – in 18 (60 %), III – in 3 (10 %) patients 5 (16.7 %) – had a history of myocardial infarction (more than 6 month) (**Table 1**).

Table 1

Characteristics of patients with CHF and AF (n=30)

Indicators	Patients with CHF and AF
Blood pressure (BP), mm Hg	
systolic (Me [25 %; 75 %])	141,50 [100; 180]
diastolic (Me [25 %; 75 %])	87,53 [70; 112]
Heart rate, contr. per min. (Me [25 %; 75 %])	77,00 [65; 98]
EF, % (Me [25 %; 75 %])	58,73 [45.38; 88]
Body mass index (BMI), kg/m ² (Me [25 %; 75 %])	29,77 [23.55; 40.9]
Arterial hypertension, n (%)	
II stage	19 (63.3 %)
III stage	5 (16.6 %)
I degree	3 (10 %)
II degree	18 (60 %)
III degree	3 (10 %)
History of myocardial infarction, n (%)	5 (16.7 %)

Patients were used treatment according to recommendations [10, 11] (**Table 2**).

Table 2

Drug therapy of patients with CHF and AF

Indicators	Main group (n=15)	Comparison group (n=15)
β -blockers, %	11 (73.3 %)	12 (80 %)
Diuretics, %	9 (60 %)	6 (40 %)
Calcium antagonists, %	3 (20 %)	2 (13.3 %)
Aspirin, %	12 (80 %)	12 (80 %)
Statins, %	6 (40 %)	8 (53.3 %)

Among the examined, 83.3 % of patients (25 people) had an elevated level of AGE (mean – 1.579 [0.884, 2.796]) (normal level – 0.9–1.1 mg/ml). The level of galectin-3 was increased in

7 patients (23.3 %) (normal level <12 ng/ml), mean – 8.542 [2.72; 17.73], which corresponds to normal values.

The obtained results showed that an increase in the FC of CHF was not accompanied by a significant change in the levels of galectin-3 and AGE, but a tendency toward an increase in the indices of galectin-3 was noted, which corresponds to the literature data (**Table 3**).

Table 3

The level of AGE and galectin-3, depending of the FC in patients with CHF and AF

	CHF and AF (n=30)	
	FC II	FC III
Galectin-3	7.987 [3.11; 16.16]	8.746 [2.72; 17.73]
AGE	1.641 [1.088; 2.796]	1.556 [0.884; 2.570]

It is known that in the development and progression of CHF plays an important role such a factor as age. In connection with this, the study of markers that determine the features of the course of CHF in different age groups is of great importance (**Table 4**).

Table 4

The level of AGE and galectin-3, depending of the age in patients with CHF and AF

	CHF and AF (n=30)		
	45–59 ages	60–74 ages	75–85 ages
Galectin-3	6.875 [3.85; 12.8]	8.293 [2.72; 16.16]	10.290* [3.87; 17.73]
AGE	1.657 [1.314; 2.120]	1.581 [0.884; 2.796]	1.516 [1.064; 2.028]

Note: * – the reliability of differences compared with the group of 45–59 years, $p < 0.05$

The analysis showed that the level of galectin-3 increases with age (by 33.18 %, $p < 0.05$), while the AGE level has no significant changes.

It is believed that the active accumulation of AGE in the blood is due to their reduced renal clearance. To clarify the role of AGE and galectin-3 in the development and progression of renal dysfunction, patients were further analysed depending on the level of GFR (**Table 5**). It should be noted that 6 patients (20 %) had normal renal function.

Table 5

The level of AGE and galectin-3 depending of GFR in patients with CHF and AF

	GFR <90≥60 ml/min./1.73m ² (n=12)	GFR <60≥30 ml/min./1.73m ² (n=12)
Galectin-3	7.670 [2.72; 17.73]	9.405 [4.1; 16.2]*
AGE	1.444 [0.884; 1.878]	1.781 [0.952; 2.796]*

Note: * – the reliability of the differences compared to the GFR group <90≥60 ml/min/1.73 m², $p < 0.05$

The results showed that with a decrease in GFR there was an increase in the level of both AGE (by 18.9 %, $p < 0.05$) and galectin-3 (by 18.44 %, $p < 0.05$). Correlation analysis revealed a close relationship between the level of AGE, serum creatinine and GFR ($r = 0.25$, $p < 0.05$ and $r = -0.22$, $p < 0.05$, respectively), while the level of galectin-3 correlated only with GFR ($r = -0.16$, $p < 0.05$).

After 3 months of treatment, the level of galectin-3 (by 21.66 %) and AGE (by 4.9 %) was decreased (**Table 6**).

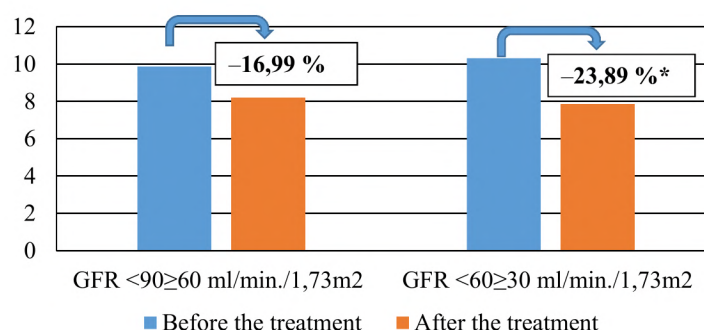
Table 6

Effect of therapy on the level of galectin-3 and AGE in patients with CHF and AF

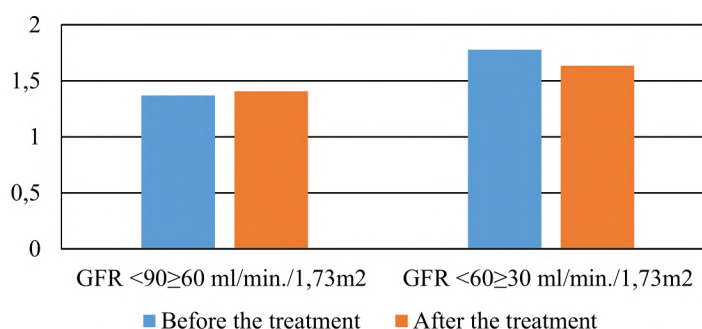
	Main group		Comparison group	
	Before the treatment	After the treatment	Before the treatment	After the treatment
Galectin-3	10.161 [4.1; 17.73]	7.960 [3.9; 14.7]	8.271 [3.85; 16.16]	5.850 [2.94; 13.48]
AGE	1.641 [0.884; 2.796]	1.559 [0.886; 2.890]	1.516 [1.078; 2.120]	1.537 [1.050; 2.340]

Against the backdrop of treatment with irbesartan, a decrease in the level of serum creatinine (by 4.89 %) and an increase in GFR (by 6.3 %) compared with the use of ACE inhibitors (by 2.9 % and 1.02 %, respectively) were detected.

The inclusion of irbesartan contributed to a significant decrease in the level of galectin-3 among patients with $\text{GFR} < 60 \geq 30 \text{ ml/min/1.73 m}^2$ ($p < 0.05$) compared with $\text{GFR} < 90 \geq 60 \text{ ml/min/1.73 m}^2$ (Fig. 1).

**Fig. 1.** Dynamics of the level of galectin-3 in patients with CHF and AF who intake irbesartan:* – the reliability of differences compared with the baseline, $p < 0.05$

Against the backdrop of treatment, there was a trend towards a decrease in the level of AGE among patients with $\text{GFR} < 60 \geq 30 \text{ ml/min/1.73 m}^2$, while there were no significant changes in patients with CHF and AF with $\text{GFR} < 90 \geq 60 \text{ ml/min/1.73 m}^2$ (Fig. 2).

**Fig. 2.** Dynamics of AGE level in patients with CHF and AF who intake irbesartan

4. Discussion

Accumulation of AGEs can be explained by certain cardiovascular changes, such as vessel stiffness, diastolic dysfunction and endothelial dysfunction. From the reports of Semba et al., it follows that a high concentration of AGE may be a risk factor for cardiovascular disease, but additional evidence is needed to confirm this statement [14, 15].

AGEs can promote the development of chronic heart failure (CHF) in two ways. First, AGEs affect the physiological properties of proteins in the extracellular matrix, creating cross-links. Secondly, AGEs causes multiple changes in blood vessels and myocardium through

interaction with AGEs receptors. AGEs can cause diastolic, systolic and vascular dysfunction through these pathways. Subsequently, these disorders can lead to the development and progression of CHF [16].

Strong evidence has been obtained in favour of the fact that the residues of AGE accumulate in the zones of vascular lesions, namely in the glomeruli of the kidneys [2]. At the same time, AGE-modified proteins break down as a result of cellular proteolysis with the formation of free products of AGE and are excreted in the urine, and therefore, in the presence of uremia is noted their active accumulation. Several mechanisms underlying the renal dysfunction have been proposed, for example, reduced renal perfusion, atherosclerosis and inflammation, endothelial dysfunction and neurohormonal activation [2]. Another possible mechanism may be an increase in AGEs. Accumulation of AGE in patients with renal dysfunction can occur in two ways. First, the increase in accumulation of AGE is due to a decrease in the clearance of the AGE decomposition products. After modification or degradation in the proximal glomeruli, AGEs are ultimately purged in urine [17]. Patients with renal dysfunction have a reduced clearance and, thus, occurs accumulation of AGE. Secondly, oxidative stress is increased in patients with renal dysfunction, which also leads to an increase in AGE formation [17]. Thus, it can be assumed that AGEs are a possible mechanism underlying the renal dysfunction. However, while the concentration of AGE circulation and their potential toxicity have been extensively studied in renal failure, there is no information available in patients with CHF.

For more than 10 years, the problem of cardiovascular syndrome has been discussed in the world. A number of studies have shown the adverse effect of decreased renal function on the course and prognosis of CHF. To date, a steady trend towards the introduction in the world of unified protocols for the treatment of cardiovascular diseases has led to positive results: improving the quality of treatment of patients and reducing mortality. However, the uncertainty of specific measures for the treatment of patients with CHF with preserved ejection fraction ($EF > 45\%$) and lack of satisfactory results induce to search for new directions of treatment and determine the possibilities of existing methods of drug therapy [18].

In the recommendations of the American College of Cardiology (ACC) and the American Heart Association (AHA) in 2017 for the treatment of patients with CHF, it is recommended to achieve low blood pressure (130/80 mm Hg), which in turn raises the issue of whiter hard control of blood pressure. Particular attention in the recommendations is given to the use of angiotensin II receptor blockers [19]. There is an opinion that the ARBs have a nephroprotective effect. However, the CHARM study showed that the use of candesartan is ineffective in patients with impaired renal function. While another study IRMA 2, using irbesartan, it was noted that the drug contributed to a reduction in the occurrence of new cases of nephropathy and dialysis, and also reduced the progression of the disease [20].

5. Conclusions

1. Among the examined, the elevated level of AGE was 83.3 % of patients, galectin-3 – 23.3 %.
2. Galectin-3, unlike AGE, clearly varies depending on age (an increase of 33.18 %, $p < 0.05$) and GFR (an increase of 18.44 %, $p < 0.05$), that is a greater impact range than AGE. However, AGE varies depending on GFR in patients with CRF (an increase of 18.9 %, $p < 0.05$), which may indicate a real damage to the clearance model and the role of glycosylation in damage to kidney function.
3. Galectin-3 is a more dynamic factor, since a positive response was noted against the background of irbesartan, in contrast to AGE. Thus, it can be said that AGE is a marker that reflects the manifestations of cardiorenal syndrome, and galectin-3 is associated with age and dynamics of CHF itself.

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ENERGY HOMEOSTASIS OF THE ARTIFICIAL BLADDER IN EXPERIMENTAL CONDITIONS

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Abstract

Patients with malignant neoplasms of the bladder or severe anatomical and functional bladder abnormalities are the candidates for cystectomy and orthotopic urine diversion. The distal portion of the ileum is the most frequent anatomical organ, which is used for the formation of the urinary conduit. The formation of a urinary reservoir from the ileum that is unable to perform new functions, and mainly programmed to isolate enzymes, digest food and absorb nutrients, salts and minerals changes the energy exchange in the ileum wall under the influence of urine.

The aim of the research is the examination of energy metabolism changes in the ileum wall during the formation of artificial bladder in experimental conditions conducted on mini-pigs. Experimental studies were performed on 21 female mini-pigs with an average age of 6 to 14 months. The duration of the experiment was 12 months. An imbalance of the ATP-ADP-AMP system was observed in the transplant tissue of the graft in different postoperative periods. Changes in the level of ATP are characteristics of the development of tissue ischemia.

Results. The values of the adenosine nucleotides sum (the energy potential indicative of the respiration rate of mitochondria) and the phosphorylation index were lower at different times of observation in the transplant tissue. The energy charge value was lower to a lesser extent relative to the control.

Conclusion. The revealed changes in the level of adenosine nucleotides confirm the severity of the disturbances of the energy homeostasis in the transplant tissue at all stages of the observation.

Keywords: cancer bladder, cystectomy, ileocystoplasty, artificial bladder, energy homeostasis, energy potential, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate, ileum.

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1. Introduction

Patients with malignant neoplasms of the bladder or severe anatomical and functional abnormalities of the bladder may be candidates for cystectomy and orthotopic urine diversion [1, 2]. The aim of the reconstructive operation is to create a urinary reservoir, which is functionally ca-

pable of adapting to the bladder in periods of urine accumulation and emptying, and maintaining renal function [3, 4].

The most frequent anatomical organ used for the formation of the urinary conduit is the distal portion of the ileum [5, 6]. The intestine transplant has the properties of resorption of urine components with the development of hyperchloremic acidosis, possibly urodynamic disorders of the upper urinary tract and the progression of renal failure, which complicates the course of the long-term postoperative period in the initially severe and age-related contingent of patients [7, 8]. Metabolic acidosis develops in 15 % of patients with a formed conduit, in 50 % of patients with intestinal reservoir from the ileum, and in 80–100 % of patients with a reservoir of the large intestine [9].

The formation of a urinary reservoir from the ileac part, which is unable to perform new functions, and also programmed to isolate enzymes, digest and absorb nutrients, salts and minerals, may also change the energy exchange in the ileum wall during contact with urine. The metabolic processes in the wall of the neobladder have not been sufficiently studied during a certain period under the influence of new conditions of functioning [10, 11].

The adenylate system is an important component of energy-producing and energy-transport cell systems. The adenosine nucleotides are capable to modify cellular functions and affect the contractile activity of smooth muscle through participation in purinergic receptors [12, 13].

Thus, it can be assumed that the role in the development of overactivity and hypoactivity of the neocyst was due to the modification of the properties of enterocytes, which, in response to mechanical and chemical stimuli, are able to change their properties, like nerve cells.

2. Aim of research

The aim of investigation is the examination of energy metabolism changes in the ileum wall during the formation of artificial bladder in experimental conditions conducted on mini-pigs.

3. Materials and methods

Studies were conducted in 2017 in the department of biochemistry SI «Filatov Institute of Eye Diseases and Tissue Therapy of NAMS of Ukraine».

Scientists have proved that the organism of pigs in its anatomical and morphofunctional features is the closest to humans and ideal for studying the pathogenesis of various morphological and biochemical processes [14].

Experimental studies were performed on 21 female mini-pigs, at an average age of 6 to 14 months. The duration of the experiment was 12 months. The experimental model of the artificial bladder was reproduced by performing cystectomy in animals with ileocystoplasty in anesthesia. The biopsies were taken from the neocyst followed by biochemical studies of the macroergs at 3, 6 and 12 months after the ileocystoplasty.

The tissue samples were homogenized with 3 ml of 6 % perchloric acid in the intestinal tissue and then the neocyst of mini-pigs in a ratio of 1:7 (weight:volume) to determine the content of ATP, ADP and AMP. The resulting acidic tissue homogenate was centrifuged at 5 °C 10 min at 10.000 rpm. The resulting deproteinized supernatant was neutralized with a 1.75 M solution of trisubstituted potassium phosphate (pH 7.0–7.5). The solution stirred and cooled for 15 minutes. The neutral extract was centrifuged at 3.000 rpm for 10 minutes. A neutral supernatant was used for the assay [15].

The parameters characterizing the state of the energy exchange under the experimental conditions were calculated on the basis of the obtained data, which are:

– energy charge (EC) by the formula

$$EC = (ATP + \frac{1}{2} ADP) / (ATP + ADP + AMP);$$

– energy potential (EP) by the ratio

$$EP = ATP / ADP;$$

– thermodynamic control of respiration (TCD) by the ratio

$$\text{TCD} = \text{ADP/AMP};$$

– index of phosphorylation (IP) by the formula:

$$\text{IP} = \text{ATP}/(\text{ADP} + \text{AMP}) [16].$$

The animals were taken out of the experiment by decapitation during anesthesia, adhering to the provisions of the “European Convention for the Protection of Vertebrates used for experiments or for other scientific purposes”.

The reliability of the discrepancy (P) between the values was determined by the Student's t test. Differences were considered statistically significant at $p < 0.05$.

4. Results

The violations of energy exchange in varying degrees of severity were detected in the transplant tissue from the intestine, depending on the observation period.

The level of ATP, ADP and AMP in the tissues of the intestine (control) and “intestinal” transplant (neocyst) was examined at 3, 6 and 12 months after surgery (**Table 1**). It was observed given that adenosine nucleotides, especially ATP, occupy an important place in the energy metabolism, and ADP stimulates the respiratory chain in the tissue cells and, along with AMP, are positive allosteric regulators of enzymatic processes.

Table 1

Content of ATP in ileum mini-pigs and neocyst after transplantation (nmol/g)

Indicator	Functional role	Statistical indicator	3 months (n=21)	6 months (n=19)	12 months (n=19)
ATP	Intestine (control)	M	1.64	1.59	1.60
		m	0.12	0.13	0.11
		p	–	–	–
		%	100.0	100.0	100.0
	Neobladder	M	0.95	1.03	1.08
		m	0.06	0.07	0.06
		p	<0.001	<0.01	<0.001
		%	57.9	64.8	67.5

Note: p – the level of significance of data differences according to intestinal tissue

A decrease of ATP levels were observed in the tissue of the formal bladder compared to the intestinal wall after 3 months by 42.1 % ($p \leq 0.001$), in 6 months by 35.2 % ($p \leq 0.001$) and after 12 months by 32.5 % ($p \leq 0.001$), which testifies to the suppression of the energy homeostasis in neocyst. The progressive increase in the level of ATP was observed in the examined transplant after 12 months from the beginning of the experiment, which may indicate activation of the adaptation process in the neobladder. There was also a progressive increase in the level of ATP in the examined transplant after 12 months from the beginning of the experiment, which may indicate activation of the adaptation process of the neobladder.

Table 2 presents the content of ADP and AMP in ileum and neocyst, where is also noted the suppression of the energy producing function, according to control, but without statistically significant positive dynamics during the 12 months of the experiment. The prolonged suppression of energy homeostasis is possibly due to the toxic effect of urine on enterocytes, the difference in the programmed vital activity of cells in the intestine and vital activity in new conditions.

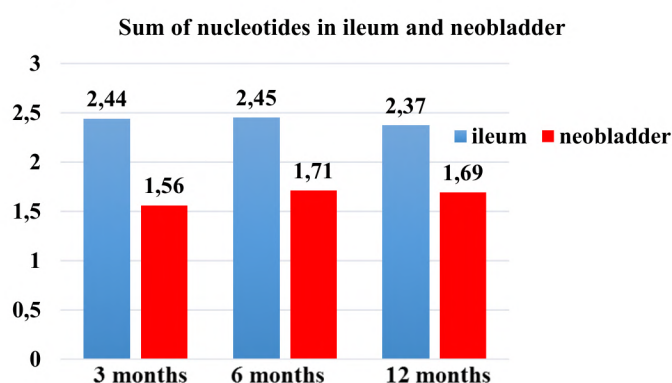
Table 2

The content of ADP and AMP in ileum mini-pigs and neocyst after transplantation (nmol/g)

Indicator	Functional role	Statistical indicator	3 months (n=21)	6 months (n=19)	12 months (n=19)
ADP	Intestine (control)	M	0.53	0.54	0.52
		m	0.03	0.04	0.03
		p	—	—	—
		%	100.0	100.0	100.0
	Neobladder	M	0.40	0.43	0.41
		m	0.02	0.02	0.03
		p	<0.01	<0.05	<0.05
		%	75.5	79.6	78.8
AMP	Intestine (control)	M	0.27	0.32	0.25
		m	0.02	0.02	0.02
		p	—	—	—
		%	100.0	100.0	100.0
	Neobladder	M	0.21	0.25	0.20
		m	0.01	0.02	0.01
		p	<0.05	<0.05	<0.05
		%	77.8	78.1	80.0

Note: p – the level of significance of data differences according to intestinal tissue

The sum of nucleotides in the ileum homogenate and neobladder in **Fig. 1**, is shown, as a decrease in energy production observed at 36.07 % ($p \leq 0.001$) after 3 month, which indicates a depression of mitochondrial respiration. Subsequent studies at 6 and 12 months did not demonstrate statistically significant changes in the sum of nucleotides compared to the primary study 3 months after the start of the experiment.

**Fig. 1.** The sum of nucleotides in ileum and neobladder ($p \leq 0.001$)

The **Fig. 2** shows the energy charge (EC) in the ileal tissue of experimental animals and neocyst during the experimental study at 3, 6 and 12 months. Energy charge is an indicator characterizing the fullness of the ATP, ADP, and AMP system with macroergic phosphate bonds. This system is considered to be filled, when its value approaches one. In such state, all adenosine phosphates are in the form of ATP. The adenosine phosphates are in the form of

AMP when its value approaches 0 and the system is not filled. The EC index is below 1 in the neocyst homogenate.

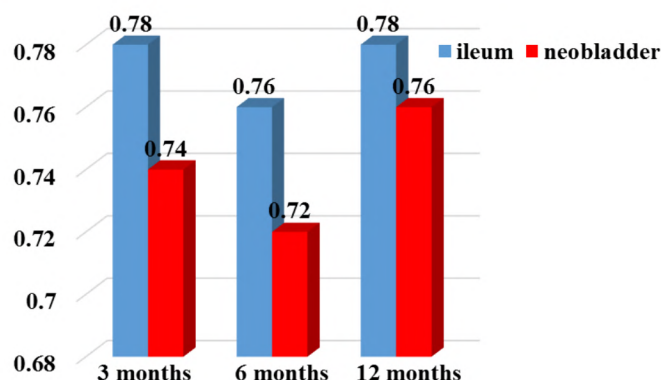


Fig. 2. Energy charge in ileum and neobladder ($p \leq 0.001$)

Energy potential (EP) is an indicator characterizing the rate of respiration of mitochondria. It is reduced by 23 % ($p \leq 0.001$) after 3 months in an artificial bladder compared to ileum. There is a slight increase in this indicator at control of 6 and 12 months, which may indicate a prospect for improving energy production. Data on the dynamics of changes in the EP are presented in Fig. 3.

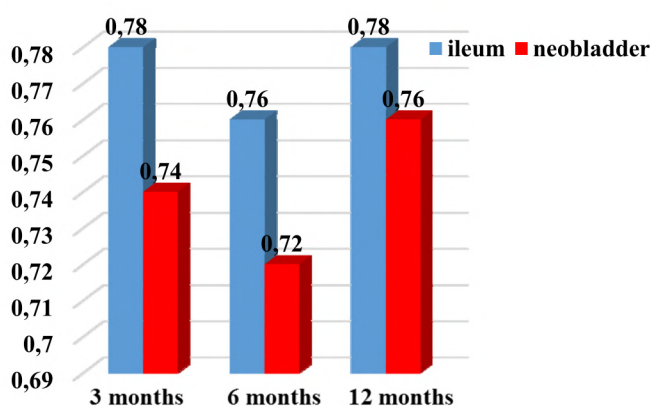


Fig. 3. Energy potential in ileum and neobladder ($p \leq 0.001$)

The data of phosphorylation index in control studies are statistically reduced by 23.9 % ($p \leq 0.001$) in 3 months in neocyst after ileocystoplasty, which testifies to the suppression of energy exchange processes. There is also a slight positive dynamics at the 12th month of the experiment. The data are provided in Fig. 4.

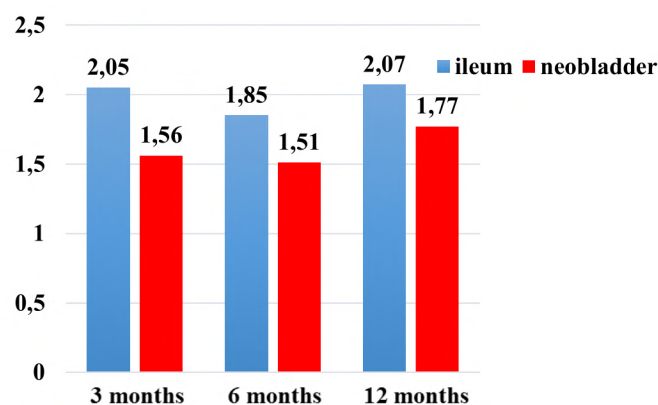


Fig. 4. The index of phosphorylation in ileum and neobladder ($p \leq 0.001$)

5. Discussion

The results of the studies performed by different authors indicate high respiratory activity of the intestinal mucosa of experimental animals, which corresponds to the intestine as a highly aerobic organ with a high intensity of energy exchange [17, 18].

There is an imbalance in the ATP-ADP-AMP system in the tissue of the transplant in different periods of postoperative observation. Changes in the level of ATP, which are characterized the development of tissue ischemia, obviously associated with an increase of macroergs in the consumption or a violation of its resynthesis in the course of creatine kinase reaction, glycolysis processes in the respiratory chain of the intestinal mucosa used as a transplant.

External irradiation also causes disturbances in energy metabolism, which can also serve as one of the causes of structural and functional damage of the small intestine [19, 20]. The values of the sum of adenosine nucleotides, the energy potential indicative of the respiration rate of mitochondria and the phosphorylation index in the transplant tissue are reduced at different times of observation according to control.

The revealed changes in the level of adenosine nucleotides confirm the severity of the disturbances of the energy homeostasis in the transplant tissue at all stages of the observation. There was also a trend towards an increase in the level of these indicators in the distant postoperative period from 3 months to 12 months of follow-up, which were still well below control. It can be explained by specific conditions of the functioning of the transplant as a bladder and the need to evaluate exogenous and endogenous factors of influence. Thus, a decrease in the level of the studied parameters was noted in the tissue of the transplant from the small intestine at different periods of observation compared with intestinal tissue.

It is still remains an issue the state of the endocrine function of the gut-transplant site and to how it manifests in the initial and in the remote observation periods. Additional clinical and functional studies can help to answer the question in what value the transplant regenerates the function of the neobladder; the degree to which the muscle tonus of its walls changes and whether the blood supply and innervation of the transplant tissue is restored to the proper degree.

6. Conclusions

1. Imbalance in the ATP-ADP-AMP system is observed in the tissue of the transplant at different periods of postoperative observation.

2. The indices of adenyl nucleotides were reduced by 36.07 %, the energy potential by 23 %, in the tissue of the transplant, and three months after the performance of enterocystoplasty.

3. It is necessary to investigate for further study the connection and influence of energy homeostasis and structural changes in the mucous ileum after the formation of the formal bladder.

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MEDICAL PSYCHOLOGICAL MOODS OF THE DOCTORS STARTING THE TRAINING AT THE QUALIFICATION IMPROVEMENT COURSES (BASED ON THE RESULTS OF THE SOCIOLOGICAL STUDY)

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Abstract

The quality of medical care depends on the training of specialists, carried out, in particular, through continuous medical education.

Aim. To form the recommendations for improving the postgraduate medical education system in the context of the overall reform of the health care system.

Materials and methods. The survey involved 823 doctors who were starting the training at the qualification improvement courses (27.40 % – males and 70.60 % – females) aged from 24 to 50+. In order to realize the purpose of the study we used, as the main method of social psychological information collecting, the method of questioning in the form of standardized (formalized) interview.

Results. Doctors of all specialties used knowledge acquired during previous courses, although with certain differences. Significant difference was found in satisfaction with the level of obtained practical skills during previous courses: 81.04 ± 1.66 % of surgeons, therapeutic, pediatric, dental specialists were very satisfied or rather satisfied, when this indicator in case of doctors of the medico-prophylactic and pharmaceutical specialties was only 39.02 ± 3.00 %. Regarding the level of theoretical knowledge, 85.51 ± 1.49 % of surgeons, therapeutic, pediatric and dental specialists were very satisfied or satisfied with the theoretical part of qualification improvement courses and 57.20 ± 3.05 % of doctors of the medico-prophylactic and pharmaceutical specialties were not satisfied or completely dissatisfied. 85.15 ± 1.50 % of doctors of surgical, therapeutic, pediatric and dental specialties completely or partly used the ideas offered during the previous courses, when 31.82 ± 2.87 % of the doctors of the medico-prophylactic and pharmaceutical specialties did not use anything in their practice. 72.42 ± 1.56 % of doctors of all specialties consider that they have not yet reached their own career maximum. Almost half of doctors of all specialties wanted to receive more theoretical and practical knowledge during the courses, although 40.15 ± 3.02 % of doctors of the medico-prophylactic and pharmaceutical specialties were interested in obtaining large volumes of information from their foreign counterparts, while only 4.29 ± 0.86 % of doctors of surgical, therapeutic, pediatric and dental specialties were interested in gaining such information.

Conclusion. The survey indicates the need to change and update the content of the qualification improvement courses work programs, as well as the creation of new ones, which is a prerequisite for the reorganization of the postgraduate medical education system. It is reasonable to create curricula and programs according to the specialties of doctors and basing on the previous survey results concerning queries and expectations.

Keywords: survey, medical postgraduate education, qualification improvement courses.

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1. Introduction

Health care is one of the most important spheres in ensuring the physical and social economic well-being and health of society. The state of this sphere can be assessed by objective indicators, which reflect the health status of the population, accessibility and quality of medical care, and largely depend on the quality of medical education, thus, the medical personnel preparedness [1].

Education is a condition for the society existence, an instrument that allows to move forward, to be updated and to change. The level of educational activity is constantly increasing, the forms and methods of education, the structure and content of educational information are continuously developing, responding to the needs of society. The importance of continuous medical education is emphasized by a number of international forums: WHO, UNESCO, the United Nations, and others [2].

In modern conditions postgraduate training of doctors is carried out according to increasing public health and society demands concerning the quality of personnel training. It is important for the doctor not only to understand his professional tasks, but also to form his orientation in public health problems according to the changing factors of the external environment, adaptation to ongoing events in life. The quality of medical care depends on the training of specialists, carried out, in particular, through continuous medical education [3].

Modern trends in the development of Ukrainian medicine require changes in doctors training and retraining. The introduction of valid technology for determining the quality of education will increase the level of doctors and pharmacists training. Recently many attempts have been made in the directions of objectification, as well as quantitative assessment of the knowledge transfer and learning, especially in health care [4–6]. Nevertheless, problematic issues of ensuring and controlling the quality of postgraduate medical education remain an urgent topic of discussion among high school staff and competent medical community [7, 8].

2. Aim of research

To form the recommendations for improving the postgraduate medical education system in the context of the overall reform of the health care system.

3. Materials and methods

The research was aimed at studying the production and social psychological aspects of the activities of doctors directed to take refresher courses at the Danylo Halytsky Lviv National Medical University. The survey involved 823 doctors starting the training at the qualification improvement courses (27.40 % – males and 70.60 % – females) aged from 24 to 50+.

The respondents had following specialties: 36.33±1.68 % – therapeutic, 18.83±1.36 % – surgical, 5.10±0.77 % – pediatric, 7.65±0.93 % – dental, 21.51±1.43 % – pharmaceutical, 10.57±1.07 % – medico-prophylactic.

Among the categories of the respondents, 12.15±1.14 % work in the village/settlement, 32.32±1.63 % – in the district center, town, 19.56±1.38 % – in the city of regional submission, 35.97±1.67 % – in the regional center.

The experience of the doctors in the specialty was: up to 5 years – 25.39±1.52 %, 5–10 years – 21.39±1.43 %, 11–15 years – 11.79±1.12 %, 16–20 years – 9.96±1.04 %, 21–30 years – 16.89±1.31 %, over 30 years – 14.58±1.23 %.

In order to realize the purpose of the study we used, as the main method of social psychological information collecting, the method of questioning in the form of standardized (formalized) interview.

In the statistical processing of the obtained data the analysis of relative and average values was carried out.

4. Results of research

First of all, we analyzed the doctors' assessment of previously completed qualification improvement courses. 74.42±1.85 % of the doctors of surgical, therapeutic, pediatric and dental specialties stated that it was possible to apply the obtained knowledge in their practice more widely.

As for doctors of medico-prophylactic and pharmaceutical specialties, knowledge gained during previous qualification improvement courses was used in their practice by 50.76±3.08 % of the respondents.

It should also be noted, that 81.04±1.66 % of surgeons, therapeutic, pediatric, dental specialists were very satisfied or rather satisfied with the practical skills acquired during the previous courses (Fig. 1).

In contrast, the percentage of satisfied or rather satisfied with the practical skills gained during previous qualification improvement courses among doctors of the medico-prophylactic and pharmaceutical specialties was only 39.02±3.00 % (Fig. 2).

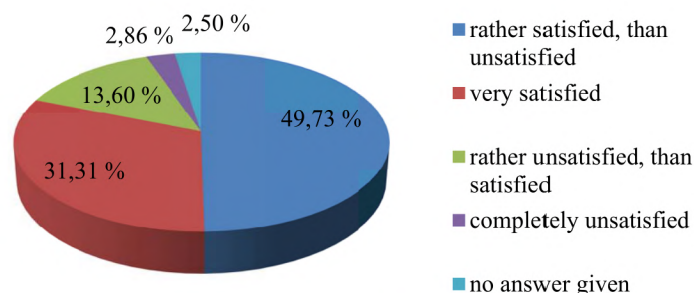


Fig. 1. Satisfaction (%) with the level of obtained practical skills during previous courses (doctors of surgical, therapeutic, pediatric and dental specialties)

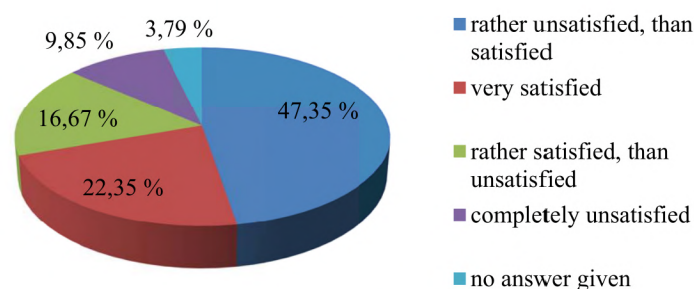


Fig. 2. Satisfaction (%) with the level of obtained practical skills during previous courses (doctors of the medico-prophylactic and pharmaceutical specialties)

Regarding the level of theoretical knowledge, 85.51 ± 1.49 % of surgeons, therapeutic, pediatric and dental specialists were very satisfied or satisfied with the theoretical part of qualification improvement courses.

Yet among doctors of the medico-prophylactic and pharmaceutical specialty 40.91 ± 3.03 % of respondents were very satisfied or satisfied and 57.20 ± 3.05 % were not satisfied or completely dissatisfied.

85.15 ± 1.50 % of doctors of surgical, therapeutic, pediatric and dental specialties completely or partly used the ideas offered during the previous courses (**Fig. 3**).

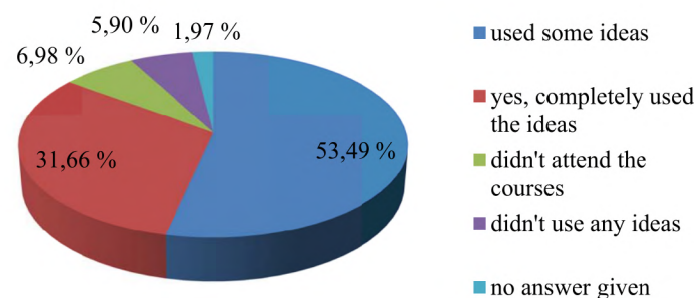


Fig. 3. Use (%) of the ideas, offered during previous courses, by doctors in their practice (doctors of surgical, therapeutic, pediatric and dental specialties)

As for the doctors of the medico-prophylactic and pharmaceutical specialties, 60.61 ± 3.01 % of the respondents completely or partly used the ideas offered during previous courses. 31.82 ± 2.87 % of the doctors of the mentioned specialties did not use anything in their practice (this indicator for doctors of surgical, therapeutic, pediatric and dental specialties was 5.90 ± 1.00 %) (**Fig. 4**).

Regarding the satisfaction of their own professional and career ambitions, 72.42 ± 1.56 % of doctors of all specialties, who began training in qualification improvement courses, stated that they have not yet reached their own career maximum.

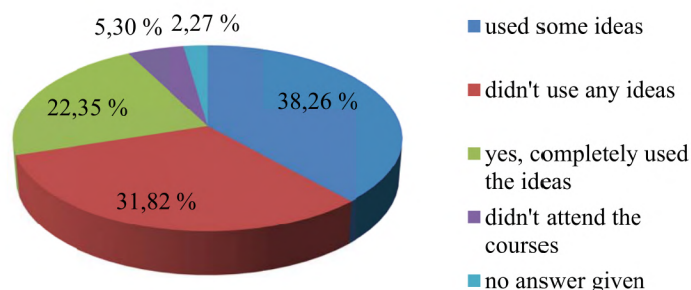


Fig. 4. Use (%) of the ideas, offered during previous courses, by doctors in their practice (doctors of the medico-prophylactic and pharmaceutical specialties)

According to the doctors of surgical, therapeutic, pediatric dental specialties, the main obstacles for their career growth include: lack of practical skills (33.63 ± 2.00 %), personal qualities (22.00 ± 1.75 %), corruption (15.92 ± 1.55 %) and the lack of theoretical knowledge (13.95 ± 1.47 %) (**Fig. 5**).

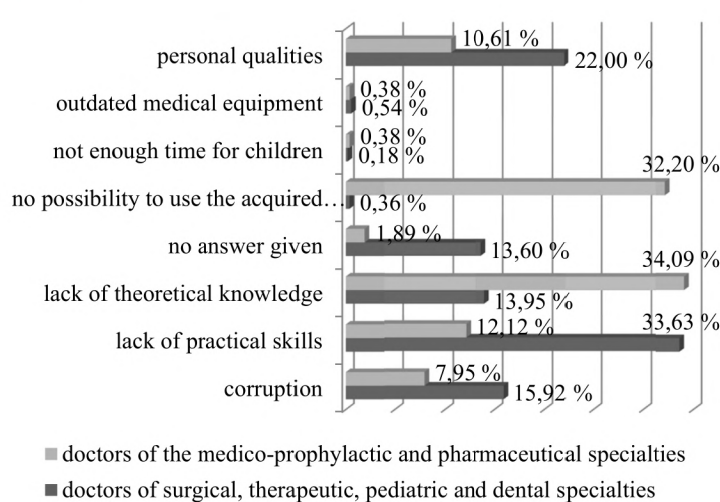


Fig. 5. Obstacles (%) for doctors' career growth (doctors of all specialties)

Doctors of the medico-prophylactic and pharmaceutical specialties placed factors hampering their career growth in the following order: lack of theoretical knowledge – 34.09 ± 2.92 %, lack of ability to use acquired knowledge – 32.20 ± 2.88 %, lack of practical skills – 12.12 ± 2.01 %, personal qualities – 10.61 ± 1.90 % (**Fig. 5**).

As for the respondents' expectations, 42.75 ± 2.09 % of doctors of surgical, therapeutic, pediatric and dental specialties wanted to receive more theoretical and practical knowledge during the courses and no more than 3.22 ± 0.75 % of doctors were interested in the information presented by foreign researchers (**Fig. 6**). Only 4.29 ± 0.86 % of the respondents believed that the subjects presented in previous qualification improvement courses were irrelevant and uninteresting. Unfortunately 46.51 ± 2.11 % of doctors of the above-mentioned specialties have evaded the question.

41.67 ± 3.03 % of doctors of the medico-prophylactic and pharmaceutical specialties were also willing to obtain more theoretical and practical knowledge. At the same time, 40.15 ± 3.02 % of respondents were interested in obtaining large volumes of information from their foreign counterparts. It should also be noted, that 12.50 ± 2.04 % of surveyed consider the topics of previous courses irrelevant and uninteresting (**Fig. 6**).

While analyzing the doctors' attitude to the prospect of changing the place of their own professional qualities realization in future, we found that 59.90 ± 1.71 % of respondents of all specialties seek to be implemented in the next 2 years in the same medical institution, where they are currently

working. However, there is a certain proportion of doctors, who seek to realize their professional qualities abroad – 17.86±1.34 % of respondents.

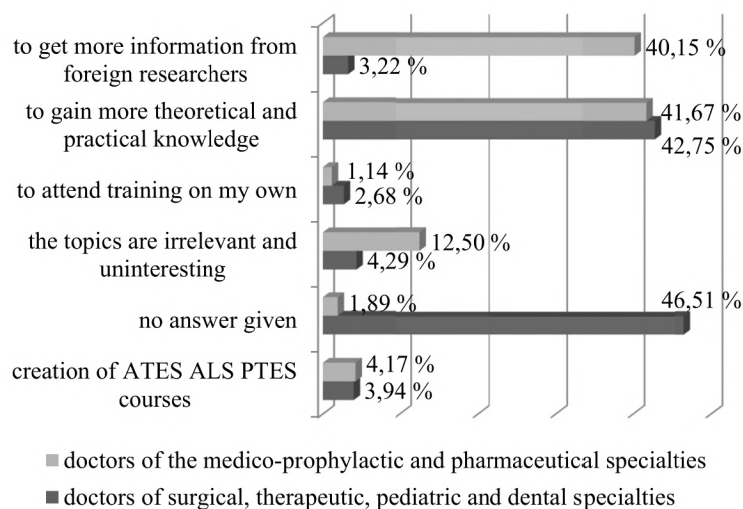


Fig. 6. Expectations (%) of doctors concerning courses (doctors of all specialties)

5. Discussion

The issue of doctors' postgraduate training effectiveness improvement and the ways of its implementation is actively discussed in modern scientific literature.

Cookson J. [9] gives 2 different points of view on how postgraduate learning process should be conducted in order to become as much effective as possible. The author presents the opinion of Dennick R. [10], who considers that a learner-centered approach is of particular importance, while identifying and building upon previous knowledge, of relating learning to context and of reflecting upon experience. Kirkham D. and Baker P. [11] come from a different perspective. Based on a requirement for a formal generic teaching program their proposals are for teaching sessions, which should be in protected time and compulsory. They outline a model syllabus around a number of common patient presentations and some more general topics.

Ali I. S. et al. [12] conducted a research, similar to ours, aiming to determine the feedback of postgraduate trainees on their current teaching methods, their level of satisfaction and preferences. The research showed that the most commonly used method of teaching was lecture, while task oriented assessment of clinical skills was the most preferred method amongst trainees, but the least practiced.

In our opinion, the realization of the postgraduate education system optimization is the most effective in terms of taking into account the needs, remarks and suggestions of practicing physicians taking the training courses.

Researchers emphasize the importance of qualification improvement courses listeners questioning, as this practice ensures the monitoring of the educational services quality and the responsiveness to the demands of the listeners [13].

It should be noted that ¾ of the respondents had more than 5 years of work experience in their specialty, reflecting the professional status of the formed specialist. This established fact becomes important in context of the professional experience continuous improvement in the chosen specialty [14].

It is assumed that the system of attestation stimulates the growth of specialists' skills, increases personal responsibility in the performance of their functional duties [15, 16].

As the results of our research showed, doctors of all specialties used knowledge acquired during previous courses, although with certain differences.

More significant differences were observed in the assessment of acquired practical skills. Doctors of surgical, therapeutic, pediatric and dental specialties appreciated the practical component of previous qualification improvement courses. Another opinion was given by

doctors of the medico-prophylactic and pharmaceutical specialties – the percentage of satisfied or rather satisfied with the practical skills gained during previous courses were almost two times less.

A similar situation can be noted in the case of obtained theoretical knowledge. Doctors, who work directly with patients, valued higher the level of gained theoretical knowledge. On the other hand, more than half of the doctors of the medico-prophylactic and pharmaceutical specialties were not satisfied or completely unsatisfied with the theoretical component of previous qualification improvement courses.

The important goal of the postgraduate medical education system is to encourage doctors to professional development, one of the vectors of which is the use and implementation of the ideas offered during courses. The practical orientation of modern postgraduate education provides its orientation to the real, practical daily application of the acquired knowledge and skills [17].

The analysis of the survey results showed that the vast majority of doctors of the surgical, therapeutic, pediatric and dental specialties completely or partly used the ideas offered during previous courses in their practice. As for doctors of the medico-prophylactic and pharmaceutical specialties, about half of the respondents completely or partly used the ideas offered during previous courses. However, one third of doctors of these specialties did not use anything in their practice.

One of the main conditions of the interesting education in the system of post-graduate medical education is the satisfaction of own professional and career ambitions. Satisfaction with work is considered in such studies as a motivation resultant influence of various external factors, which, refracting in the minds of respondents through the motivational core, goals and values, form the attitude to work and its individual parts [18].

Most physicians of all specialties, who have begun training in qualification improvement courses, believed that they have not yet reached their own career maximum. This personal social psychological factor should be used as one of the encouraging motives for raising the level of interest during training.

Comparison of the results presented above showed, that while planning the course for the doctors of surgical, therapeutic, pediatric and dental specialties more attention should be paid to the development of practical skills, and provision of modern theoretical knowledge should be emphasized for the doctors of the medico-prophylactic and pharmaceutical specialties.

Updating existing and creating new programs depends to a great extent on the expectations of the listeners. As the survey showed, doctors of different specialties come to training with different expectations.

About half of the doctors of all specialties wanted to receive more theoretical and practical knowledge during the courses, but the doctors of surgical, therapeutic, pediatric and dental specialties mainly were not very interested in the information of foreign researchers. All topics presented in previous qualification improvement courses were relevant and interesting for doctors of the above-mentioned specialties.

In a similar study [19] among doctors of dental specialty, who started qualification improvement courses, it was discovered that most of them (78 %) tried to use the situation to “learn something new”, “discuss professional issues with colleagues”, “see how others work”, “get acquainted with their colleagues”, “make sure that they work correctly”, “rest from routine work”. 95 % of the listeners preferred practical classes at the bases of the department and didn’t see any benefits from classes in the auditorium, and only 5 % wished to stay at lectures.

About half of doctors of the medico-prophylactic and pharmaceutical specialties were interested in obtaining information from their overseas colleagues in large volumes. Apparently, this fact is related with their assessment of previous qualification improvement courses, because a relatively higher percentage of respondents stated that the subjects of the previous courses were not relevant and not interesting.

Relevant results were also obtained during a study [20] conducted in 2010–2014 among family doctors who were undergoing thematic improvement courses. It was determined that for optimization and professional improvement of family doctors work it is necessary to review the thematic filling of the training cycles. Cycles of thematic and pre-certification education for family physicians should include a large block of information material with practical tasks, in particular, based on the safety of drug therapy.

More than half of all doctors wanted to be implemented in the next 2 years in the same medical institution, where they are working at the moment, yet almost a quarter of the respondents considered a possibility to realize their professional qualities abroad.

6. Conclusion

1. As our research showed, the system of postgraduate medical education forms new skills for the listeners of qualification improvement courses, promotes becoming new theoretical knowledge, encourages the implementation of ideas offered during the courses in practice. However, the results of the survey determine the direction of the postgraduate medical education system reforming.

2. Actual social psychological survey indicates the need to change and update the content of the work programs of the qualification improvement courses, as well as to create new ones, which is a prerequisite for the reorganization of the postgraduate medical education system. In particular, a significant increase is needed in the variational component of the listeners' choice including corresponding relevant content.

3. It is necessary to provide a preliminary study on the inquiries and expectations of doctors directed to qualification improvement courses in order to correct existing curricula and to prepare new ones.

4. We consider differentiation of doctors in specialties as an expedient step. In particular, the doctors of surgical, therapeutic, pediatric and dental specialties, and medico-prophylactic and pharmaceutical should be united into separate groups.

5. It is reasonable to create curricula and programs according to the specialties of doctors and basing on the previous survey results concerning queries and expectations. Moreover, it should be taken into consideration, that doctors of surgical, therapeutic, pediatric and dental specialties require more practical training and doctors of medico-prophylactic and pharmaceutical specialties require more scientific and theoretical one.

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THE STUDY OF THE LEVELS OF METALLOPROTEINASES, CYTOKINES AND LYMPHOCYTE ACTIVATION MARKERS IN SEMINAL PLASMA OF MEN, DEPENDING ON FERTILITY

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Abstract

The study of the concentration of metalloproteinases, pro- and anti-inflammatory cytokines, lymphocyte activation markers in seminal plasma of men with oligosymptomatic forms of chronic inflammation of the urogenital tract (CIUT), depending on the fertility rate are presented in the article.

Quantitative characteristics in male sperm with different forms of pathospermia were studied for matrix metalloproteinases (MMP)-2 and MMP-9 and their inhibitors – tissue inhibitors of metalloproteinases (TIMP)-1 and TIMP-2. It was shown, that during chronic inflammation of the urogenital tract of men are shifting levels of cytokine profile, reducing the concentration of metalloproteinase-2, chemokines – fractalkine and regulated by activation, expression and secretion of normal T-cells (RANTES), sharp increase in IL-8, MCP-1 and elevation of the CD25+/CD95+, indicating that the disturbance of apoptosis of pathological forms generative cells and their accumulation in the sperm.

The ratio of immunological indices IL-2/IL-4, IL-10/IL-12 was calculated and a significant increase in the IL-10/IL-12 index was noted in the group of individuals with elevated levels of hypercapitated form of sperm, and a reduced proportion of this ratio was observed in the microsome morphology of sperm. Prolonged inflammation in the genital area accompanied by depletion of the local immune system, resulting in the development of infertility.

Immunocorrection therapy for men with CIUT should take into account the peculiarities of changes in local immunity and be differentiated depending on the prevalence of certain pathological forms of sperm and changes in the cytokine profile of the seminal plasma.

Keywords: male infertility, sperm, seminal plasma, metalloproteinase, cytokine profile, markers of lymphocyte activation.

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1. Introduction

One of the main directions of modern scientific research is the study of proteolysis as a special form of biological regulation. Proteolysis is constantly occurring in living organisms and in the environment under the influence of microorganisms, resulting in a variety of biologically active substances – enzymes, hormones, peptides, amino acids, etc. Proteolytic enzymes that have high biological activity are involved into the functioning of various organs and systems of the body and in the regulation of biological processes. They not only carry out non-specific decomposition of protein molecules, but also control the functions and systems of the organism, which is realized in the reactions of general and limited proteolysis. According to modern notions, protease enzymes support the balance between death and cell degradation and their recovery, they participate in cellular mechanisms of proteasome degradation [1]. The proteolytic processes are necessary for the normal functioning of the organism. Violations of the mechanisms of biological regulation can cause serious diseases: arthritis, multiple sclerosis, cancerous tumors, and others. Proteolytic enzymes play an important role in metastasis in the passage of malignant cells through the membrane, their migration and invasion into the extracellular matrix. In these processes, 4 classes of

proteolytic enzymes – matrix metalloproteinases, cysteine and serine proteinases, as well as protein kinase aspartate of cathepsin D – participate [2, 3]. Matrix metalloproteinases (MMP) – the family of extracellular zinc-containing proteinases. Today about 30 MMPs are known, their role in the processes of morphogenesis, apoptosis, remodeling and resorption of tissues continues to be studied [4, 5]. There are five types of MMP: collagenase (MMP-1, -8 and -13), glutathione (MMP-2 and -9), stromelysin (MMP-3, -10, -11), elastase (MMP-7, -12) and the membrane type (MT-MMP, MMP-14, -15, -16 and -17) [6, 7].

MMPs are secreted as well as inflammatory and stromal cells in response to exogenous stimuli and proinflammatory cytokines: tumor necrosis factor (TNF)- α , interleukin (IL)-1 β . Such cytokines, such as interferon (IFN)- γ , IL-4 and IL-10, decrease the secretion of MMP [8, 9].

The substrate for MMP-2 and MMP-9 is collagen I, IV, V, VII and X types, elastin, fibronectin, IL-1 β , IL-2, IL-8, IL-15, transforming growth factor (TGF)- β , chemokines, INF- β , soluble forms of receptors for a number of interleukins, etc. The works of scientists has determined the effect of MMP-3, MMP-8, MMP-13, MMP-14, MMP-28 on the level of several chemokines: fractalkine, IL-8, regulated by activation, expression and secretion of normal T cells (RANTES) [10, 11]. Consequently, metalloproteinases are involved into the development of the inflammatory process [12, 13]. Protective functions for MMP-9 were revealed in the suppression of lymphoproliferation and dampening of systemic autoimmunity, cautioning against the long-term use of MMP inhibitors in autoimmune lymphoproliferative syndrome [14]. MMP2 and MMP9 lay a key role in regulating T-cell activation [15]. MMP activity is regulated by tissue inhibitors of metalloproteinases (TIMP) [16, 17]. The role of MMP in the functioning of the male reproductive system and in the fertilizing ability of sperm [18] was investigated. The raised levels of MMP-2 and MMP-9 in the secretion of prostate and testicles in men with prostate hyperplasia were demonstrated [19]. Quantitative characteristics in male sperm with different forms of pathospermia were studied for MMP-2 and MMP-9 and their inhibitors TIMP-1 and TIMP-2 [20]. It is believed that TIMP-1 is specific for MMP-9, and TIMP-2 for MMP-2 [10]. It is clear that during the inflammatory process in the urogenital tract of men, the levels of cytokines and biological substances that regulate their functional activity undergo certain changes. In men with chronic urogenital infection, a significant increase in the concentration of a number of cytokines - IL-1 β , IL-5, IL-6, IL-13, IL-15, IL-17, IL-23, INF- α , monocyte chemotactic factor (MCP) and to a lesser extent - IL-2, IL-10, IL-12, IL-18, TNF- α , INF- γ [21]. The elevated levels of these inflammatory mediators indicate the involvement of immune factors to protect the male genital tract. These changes are more important in the development of complications, in particular, infertility. The study of the peculiarities of the interaction of cytokines and metalloproteinases during inflammation in male genitals, obviously, will have not only diagnostic and therapeutic value, but may also address the issue of increasing the fertilizing potential [11].

2. Aim of the research

The objective of this study was to investigate the pro- and antiinflammatory cytokines in the seminal plasma of men with chronic inflammation of the urogenital tract (CIUT), CD4+CD25+, CD4+HLA-DR4+ and CD4+CD95+ lymphocytes activation markers, MMP-2 metalloproteinase, MMP-9 and their inhibitors TIMP-1, TIMP-2 depending on fertility.

3. Materials and methods

The research was conducted on the basis of the Department of Family Medicine and Outpatient Clinical Care and the Research Laboratory of the National Medical Academy of Postgraduate Education named after P. Shupik from 2013 to 2016.

Under our supervision were 69 patients with oligosymptomatic forms of chronic inflammation of the urogenital tract (CIUT) aged from 24 to 40 years. All subjects were divided into two groups: the first (35 people) – infertile men and the second (34 people) – fertile men. All patients were examined clinically, including examination and palpation of the penis, scrotum and prostate gland, and carefully collected anamnesis. All patients gave semen for analysis. The ejaculation should not occur within 4–5 days on the eve of the delivery. Semen analysis was performed half an

hour after ejaculation and in accordance with WHO guidelines. Considered the concentration of sperm, pH of semen, the content of leukocytes and their populations (granulocytes, macrophages, lymphocytes). Patients with leucospermia were not included in the study. To determine the morphology staining for Papanicolaou was used.

The levels of the following cytokines in serum and seminal plasma were studied by immunoassay in laboratory equipment of Sanofi diagnostic Pasteur (France) using ProCon (Protein Contour, SPb) certified test systems in Ukraine: IL-1 β , IL-2, IL-4, IL-6, IL-10, FNP- α . The concentration of IFN- γ was studied using the Ukrainian-certified test systems of the diagnostic systems (RF – Nizhny Novgorod). IL-8 and MCP-1 cytokine levels were tested in serum of blood and serum from semen by an immuno-enzymatic method using the IFA-BEST test system of LLC Vector-Best, Saint-Petersburg, level RANTES (regulated by activation, expression and secretion of normal T cells) and fractalkine test systems of RayBiotech, Inc., USA. The concentrations of IL-12 and IL-18 cytokines in serum and seminal plasma were determined by immunoassay using the STAT-FAX-303 PLUS analyzer (USA) at a wavelength of 492 nm. To determine the level of lymphocytes that expressed on their surface receptors for functional activation of CD4+CD25+, CD4+CD95+, CD4 + HLA-DR4 + (TRAIL receptor 1) cells, was used an erythrocytic diagnosticum with monoclonal antibodies produced by the MedBioProject (RF) – Moscow) The ratio of immunological indices IL-2/IL-4, IL-10/IL-12 and CD25+/CD95+ was calculated.

The levels of MMP-2, MMP-9, TIMP-1 and TIMP-2 were measured in serum from sperm by the immuno-enzymatic method using the Ameters int. (United Kingdom) Biotrak test system.

Control parameters of concentrations of all of the above-mentioned cytokines were obtained during the study of 24 fertile men without clinical signs of the disease, similar in composition according to age.

Statistical analysis was performed on a personal computer using the MedStat program package [22]. At the same time criterion χ^2 was used for checking the indicators for normal distribution. The median was calculated, 25 % quartile, 75 % quartile, confidence intervals. To compare the indicators, the criterion χ^2 , the two-sided critical region, was used. To analyze the presence and the strength of the relationship, the Kendal correlation coefficient τ was calculated.

4. Results

The study of levels of metalloproteinases MMP-2, MMP-9 and their inhibitors TIMP-1, TIMP-2 in serum of male sperm with CIUT showed that the level of MMP-2 was significantly lowered compared to control values. However, the MMP-2 in the group of infertility men declined more significantly – the difference of 2.9, in the group of fertile men the multiplicity of the difference was 1.4. The levels of MMP-9, TIMP-1 and TIMP-2 did not differ from the normative (**Table 1**).

Table 1

Indicators of levels of metalloproteinases and their serum plasma inhibitors in men with CIUT (Me \pm m [95 % confidence interval])

Indexes	Norm	Infertile men	Fertile men
MMP-2, ng/ml	1646.4 \pm 124.8 [1351.2; 1967.4]	576.489.3 \pm *** [351.3; 767.6]	1134.478.6 \pm ** [993.7; 1349.8]
MMP-9, ng/ml	47.49.7 \pm [21.2; 77.4]	44.98.5 \pm [29.1; 67.7]	45.57.9 \pm [31.8; 68.5]
TIMP-1, ng/ml	23478.3675.4 \pm [21751.2; 24867.4]	27547.6704.7 \pm [25424.5; 28667.6]	24163.6694.1 \pm [21974.3; 2589.7]
TIMP-2, ng/ml	4538.2358.5 \pm [3851.2; 5367.4]	4794.5289.9 \pm [4021.6; 5167.8]	4635.9311.5 \pm [3905.7; 5397.5]

Note: the reliability of the difference between the indicators of the group and the indicators taken at the norm at values P: * – <0,05, ** – <0,01, *** – <0,001

The results of the study of the cytokine profile indicate that in the examined patients with infertility there is a significant increase in the concentration of IL-8 in the seminal plasma (215.5 ± 7.8 pg/ml, $p < 0.01$) and the monocytic chemotactic factor MCP (926.4 ± 30.2 pg/ml, $p < 0.001$), in the group of fertile men the levels of these cytokines also increased (85.5 ± 5.7 pg/ml, $p < 0.01$ and 185.2 ± 12.5 pg/ml, $p < 0.001$, respectively), but the multiplicity of the difference was significantly lower compared to infertile men (**Table 2**). So for IL-8, the multiplicity of difference in the group of infertility and in the group of fertile men was 6.0 and 2.4 respectively, for MCP – 11.2 and 2.2.

Table 2Immunological parameters of male sperm serum with CIUT (Me \pm m [95 % confidence interval])

Indexes	Norm	Infertile men	Fertile men
IL-1 β , pg/ml	54.6 \pm 0.8 [51.2;67.4]	5.4 \pm 0.5 *** [5.1;6.3]	36.4 \pm 2.1** [31.3;39.8]
IL-2, pg/ml	86.5 \pm 1.7 [63.4;89.5]	14.2 \pm 1.3*** [12.5;16.5]	47.7 \pm 5.1** [35.2;64.6]
IL-4, pg/ml	24.1 \pm 1.2 [23.5;32.7]	24.1 \pm 0.5 [23.1;25.6]	25.1 \pm 0.9 [23.1;27.6]
IL-6, pg/ml	34.1 \pm 1.2 [32.1;36.7]	32.2 \pm 1.4 [30.2;35.7]	32.0 \pm 1.3 [30.1;34.5]
IL-8, pg/ml	36.1 \pm 1.5 [35.4;40.3]	215.5 \pm 7.8*** [197.0;237.7]	85.5 \pm 5.7** [69.4;107.5]
IL-10, pg/ml	36.3 \pm 1.3 [32.2;39.4]	35.1 \pm 1.3 [32.5;41.5]	35.6 \pm 1.5 [32.4;40.1]
IL-12, pg/ml	13.6 \pm 1.4 [11.8;15.8]	9.6 \pm 0.3* [8.3;10.2]	13.5 \pm 0.8 [11.5;15.7]
IL-18, pg/ml	28.1 \pm 0.8 [25.6;30.4]	21.0 \pm 0.6*** [16.8;22.5]	25.5 \pm 1.2* [22.6;27.1]
IL-2/ IL-4	3.5 \pm 0.3 [2.9;5.1]	0.6 \pm 0.2*** [0.4;1.7]	1.9 \pm 0.3** [1.4;2.6]
IL-10/ IL-12	2.7 \pm 0.2 [2.2;5.4]	3.7 \pm 0.32** [2.0;6.7]	2.6 \pm 0.27 [2.1;4.1]
MCP, pg/ml	82.4 \pm 1.4 [78.8;80.3]	926.4 \pm 30.2*** [845.1;969.6]	185.2 \pm 12.5*** [886.0;992.8]
TNF- α , pg/ml	12.9 \pm 0.5 [11.2;15.9]	12.1 \pm 0.9 [11.5;15.2]	11.9 \pm 1.0 [11.0;14.7]
RANTES, pg/ml	40.5 \pm 2.2 [37.6;43.5]	12.7 \pm 1.0*** [9.8;14.6]	45.8 \pm 2.3* [40.1;49.7]
Fractalkine, pg/ml	29.4 \pm 1.5 [26.1;32.0]	16.3 \pm 1.3*** [12.9;18.4]	87.4 \pm 8.2*** [73.6;108.0]
INF- γ , pg/ml	5.3 \pm 0.2 [5.0;5.7]	5.1 \pm 1.2 [4.3;7.1]	5.0 \pm 0.8 [3.5;7.3]
CD4+CD25+, %	10.7 \pm 1.5 [9.3;14.4]	16.3 \pm 1.4** [12.5;19.6]	12.4 \pm 1.3 [10.2;15.6]
CD4+CD95+, %	11.8 \pm 1.2 [8.1;14.0]	17.9 \pm 1.3** [10.3;21.1]	24.2 \pm 1.5*** [20.1;28.6]
CD4+HLA-DR4+, %	1.2 \pm 0.2 [0.9;1.4]	1.8 \pm 0.2* [1.4;1.9]	1.3 \pm 0.2 [0.9;1.7]
CD25+/CD95+	0.900.14 \pm [0.80;1.12]	0.970.12 \pm * [0.96;1.22]	0.510.11 \pm * [0.39;0.65]

Note: the reliability of the difference between the indicators of the group of infertility men and the rates taken for the norm at values P: * – $< 0,05$; ** – $< 0,01$; *** – $< 0,001$

At the same time, in infertile men observed a significant decrease in the serum plasma concentrations of IL-1 β (5.4 ± 0.5 pg/ml, $p < 0.01$), IL-2 (14.2 ± 1.3 pg/ml, $p < 0.01$), IL-12 (9.6 ± 0.3 pg/ml, $p < 0.01$), IL-18 (21.0 ± 0.6 pg/ml, $p < 0.01$). In the group of fertile men, the abovementioned cytokines also decreased, but less importantly than in infertile men. The concentrations of TNF- α , IL-4, IL-6, IL-10, IFN- γ in the seminal plasma of both groups of men did not exceed the corresponding mean concentrations of healthy men. Moreover, the levels of concentration of all of the abovementioned cytokines in the peripheral blood of patients with CIUT varied within normal limits. Interestingly, the study of the IL-10/IL-12 ratio was quite interesting. The literature indicates that a decrease in the IL-10/IL-12 ratio is a key factor in suppressing the effectiveness of local immune defense in the lower sections of the urogenital tract, inhibition of cellular mediated immune response, and the development of immunosuppression [23, 24]. In our study, there were diverse shifts in this ratio of infertility men. A significant increase in the IL-10/IL-12 index was noted in the group of individuals with elevated levels of hypercapitated form of sperm, and a reduced proportion of this ratio was observed in the microsomatic morphology of sperm. The obtained data emphasize the importance of microenvironment in the process of maturation of gametes. In the group of infertile men, the concentration of RANTES was significantly lower than normal (12.7 ± 1.0 pg/ml, $p < 0.05$). And the multiplicity of the difference on average was 3.2 times. However, in part of the patients of the infertile group (14 people) the difference in frequency was 6.3 times (mean 6.4 ± 0.8 pg/ml, $p < 0.001$). Morphometry of the sperm in this group of patients showed hypercapitated morphotype of sperm ($r = -0.86$, $p = 0.4$). The level of fractakline in the serum of semen of infertile patients significantly decreased (16.3 ± 1.3 pg/ml, $p < 0.001$). In the group of fertile men, the levels of RANTES and fractakline, on the contrary, increased (45.8 ± 2.3 g/ml, $p < 0.05$ and 87.4 ± 8.2 pg/ml, $p < 0.001$, respectively).

In patients with CIUT, which was complicated by infertility, there was a marked increase in CD4+CD25+ levels in sperm, both in relative and absolute values and was 16.3 ± 1.4 %, $p < 0.01$ (10.7 ± 1.5 % in the control group), ($p < 0.05$), indicating an increased activity against infectious defense. At the same time, the mean value of CD4+CD25+ in semen was not changed in fertile men. The level of lymphocytes in the sperm of the infertile men with CD4+CD95+ expression was elevated in compare to the control group in both relative and absolute values and was 17.9 ± 1.3 %, $p < 0.01$ (11.8 ± 1.2 % in the control group). The frequency difference was 1.5 times. At the same time, some of the patients in this group (12 people), the level of CD4+CD95+ in the sperm, on the contrary, decreased with respect to control, and the average value for this part of the patients was 9.0 ± 1.1 %, ($p < 0.01$), which was less than 1,3 times the adopted norm. Decreased immunocompetent cells ready to enter the process of apoptosis can lead to imbalances in the immune system. About the activation of anti-infective protection is the tendency to increase the absolute level of cells with expression of CD4+HLA-DR4+ in this group of patients. In view of the literature data [25], we calculated the ratio of CD25+/CD95+. The study showed that there is a positive correlation between the increased value of the proportion of CD25+/CD95+ and the presence of hypercapitated forms of sperm. At the same time, an increase in this index was observed in the group of infertile men. A significant decrease in the proportion of CD25+/CD95+ was observed in a group of infertile men, whose spermometry has established the predominance of normosomatic forms of sperm. The obtained data, in our opinion, testify to the toxic effect of activated lymphocytes in the sperm against the suppression of the processes of apoptosis.

5. Discussion

The results of our study showed that the development of such a complication in men, as infertility on the background of chronic inflammation of the urogenital tract is accompanied not only by the shift of microenvironment, but also by pathomorphological changes in the generative cells. The literature notes that MMP-2 inactivates a number of chemokines – fractakline, RANTES and proinflammatory cytokines – IL-1 β , TNF- β [26]. The IL-8 protein under the influence of MMP-9, on the other hand, leads to increased activity of this cytokine [11]. MMP-9 by inactivating INF- γ can lead to prolonged inflammation [7]. Inflammation is usually characterized by elevated levels of metalloproteinases [9], however, the male examination from CIUT indicates, on the contrary,

a decrease in MMP-2 levels and a normal level of MMP-9 in serum, along with lower concentrations of proinflammatory cytokines IL-1 β , IL-2, IL-12, IL-18, which may indicate an exhaustion of the local immune system, despite normal immunological parameters in systemic circulation. The altered parameters of the cytokine profile in the urogenital tract obviously adversely affect the processes of maturation, which results in the prevalence of pathological forms of sperm in the group of infertile men. Thus, the determination of the lower concentration of MMP-2 in serum can be a negative predictor of the development of infertility.

6. Conclusions

1. During the chronic inflammation in the urogenital tract, men undergo changes in pro- and anti-inflammatory cytokine levels, decreased concentrations of MMP-2, chemokines-fractalkine and RANTES, a sharp increase in IL-8, MCP-1 and an increase in the CD25+/CD95+ index, indicating on violation of the processes of apoptosis of pathological forms of the generative cells, which leads to their accumulation in the semen.

2. Long inflammatory process in the genital organs is accompanied by exhaustion of the local immune system, resulting in the development of infertility.

3. Immunocorrection therapy for men with CIUT should take into account the peculiarities of changes in local immunity and be differentiated depending on the prevalence of certain pathological forms of sperm and changes in the cytokine profile of the seminal plasma.

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SUBSTANTIATION FOR THE TECHNOLOGY OF OBTAINING ANTIMICROBIAL SPRAY ON THE BASIS OF SILVER AND COPPER CITRATES

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Abstract

Formation and development of the veterinary pharmacy and veterinary drugs market in Ukraine is an urgent issue. In order to prevent infection of the mammary gland in cows after milking, the nipples are treated with a suitable disinfectant for complete immersion or spraying. However, the agents for the prevention of mastitis, presented in the Ukrainian market, are expensive and there is a need to develop domestic veterinary preparations of different forms of release.

This work is a continuation of research on the development of the composition and technology of a means for antiseptic treatment of nipples and udders of cattle, in the form of a spray, in order to prevent microbial contamination and prevent mastitis.

To obtain a stable veterinary drug based on citrate of silver and copper, the dissolution regimes of the substances were investigated, the order of introduction of the active ingredients and auxiliary substances into the solution, the duration and the rate of stirring during dissolution. It is established that the most optimal are the following technological modes of spray preparation: dissolution temperature 20–22 °C, mixing time from 10 to 15 minutes, with stirring intensity from 150–200 sec⁻¹. The selected technological regime ensures the stability of the veterinary drug “Argocide-copper” during storage for 12 months. The technology of obtaining a spray in the conditions of industrial production of veterinary preparations of LLC “Brovafarm” (Brovary, Ukraine) is developed. Critical stages and critical control points in the production process of a veterinary preparation of antiseptic action are determined.

Keywords: silver citrate, copper citrate, spray, antiseptic action, prevention of mastitis.

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1. Introduction

An important task of modern pharmaceutical technology is the creation of effective and safe medicines, including veterinary pharmacy. Formation and development of the veterinary pharmacy and veterinary drugs market in Ukraine is an urgent issue, which is connected with modern processes in society [1]. The problem of mastitis in productive animals is urgent. The content of cattle on a limited area of farms leads to an increase in microbial contamination and an increase in the virulent properties of the opportunistic bacterial flora. High milk production causes the intense functioning of the mammary gland, accompanied by a decrease in organ resistance, resulting in inflammation [2]. Therefore, growth the importance of veterinary and sanitary measures, especially the prevention and treatment of breast diseases of cattle [3]. At the current stage of livestock development, there is no one agro-industrial economy of Ukraine that would have bypassed the problem of mastitis cattle. Mastitis causes considerable economic damage for all countries of the world. The main costs include a decrease in the dairy productivity of animals [4].

In the treatment of mastitis in cows, antibiotic solutions for parenteral administration and suspension in syringe tubes for intracisternal use are most often used [5]. Modern anti-mastics are also produced by pharmaceutical factories in the form of gels, ointments, emulsions, suspensions and solutions for external use, etc. [6]. According to the State Veterinary

and Phytosanitary Service of Ukraine, as of 2017, 46 trade names for cattle mastitis products were registered on the veterinary drugs market of the country [7]. But, experience shows that the problem of improving dairy herds from mastitis is not always solved by medical measures, they must be supported by a set of organizational, economic, zootechnical, veterinary and sanitary measures aimed at increasing the nonspecific resistance of the organism, timely elimination of causes and factors contributing to the emergence of new cases disease [8].

In order to prevent infection of the mammary gland in cows after milking, the nipples are treated with a suitable disinfectant for complete immersion or spraying. For antiseptic treatment of the udder are used agents containing various antiseptic or disinfecting compounds, such as chlorhexidine, hydrogen peroxide, bleach, quinazole, phenol and its derivatives [9, 10]. Iodine compounds have become very popular in the prophylactic preparations for the treatment of cattle's udder [11, 12]. Disadvantages of such drugs may be low effectiveness of treatment, and the possibility of causing side effects.

It is established that the drugs for the prevention of mastitis, presented in the Ukrainian market, are costly and there is a need to develop domestic veterinary preparations of different forms of release [13].

This work is a continuation of the research on the development of the composition and technology of a means for antiseptic treatment of the nipples and udder of cattle in the form of a spray under the conventional name "Argocide-copper", with the aim of preventing microbial contamination and preventing mastitis [14, 15].

2. Aim of the research

Substantiation of the technology of obtaining an antimicrobial spray based on silver and copper citrate in industrial conditions, determination of critical stages and critical control points in the production process.

3. Materials and methods

As a research object were chosen a series of preparations with the following proportions of the components of mass %: silver citrate 15.0–30.0; copper citrate 15.0–30.0; dexpanthenol 0.5–5; polyquaternium 10 0.1–1.0; polysorbate 20 0.2–3.0; essential oil of lemon 0.01–2.0; essential oil of juniper 0.01–2.0; water purified to 100.0. The active substances – silver and copper citrate, manufactured by Nanomaterials and Nanotechnologies Ltd., Kyiv, Ukraine, D – panthenol USP (BASF SE, Germany) were used.

The quantitative content of silver ions in the spray was determined by thiocyanometry [16]. The quantitative content of copper ions was determined according to the method of the State Pharmacopoeia of Ukraine 2.0 (SPhU) "2.2.25 Absorption spectrophotometry in the ultraviolet and visible regions". Quantitative determination of dexpanthenol was carried out by liquid chromatography according to the procedure of SPhU "2.2.29. Liquid chromatography. Potentiometric determination of the pH of the experimental samples was carried out according to the procedure of SPhU 2.0, item 2.2.3. The measurements were carried out using a pH meter "Seven Easy pH" complete with electrodes manufactured by Mettler Toledo (China). The dissolution criterion was the absence of "visible particles" in the solution. Statistical processing of experimental data was carried out in accordance with the requirements of the article SPhU 2.0 "5.3.N.1. Statistical analysis of the results of a chemical experiment" [17].

4. Results

To obtain a stable veterinary drug in the form of a silver citrate and copper spray for veterinary medicine, the dissolution regimes of the substances, the order of administration of the active ingredients and auxiliary substances into the solution, the duration and rate of stirring during dissolution were investigated [18]. The order of administration of ingredients is one of the most important factors for obtaining a stable dosage form for veterinary medicine.

The calculated volume of the silver citrate and copper citrate solution was loaded into the reactor and purified water was added from the collection tank at a temperature of $(20 \pm 5)^\circ\text{C}$, then

the reactor was loaded sequentially with stirring: dexpanthenol, stirred for 10–15 minutes; polyquaternium – 10, mixed for 15–20 minutes. Lemon and juniper oil were mixed in a separate container with a calculated amount of polysorbate 20, and then added to the stock solution, mixing until completely dissolved.

At the same time, technological parameters were processed, including the temperature and time regimes, the duration and speed of mixing during the preparation of the solution. Since the stability of the solution of citrate of silver and copper depends on the temperature, and the solution of silver citrate is not recommended to be heated to a temperature above 500 °C, so the preparation of the solution should be carried out at a temperature of 20–22 °C.

We studied the dissolution rate of the components at the chosen temperature regime. Intensity – the speed of achieving the required technological result - the time spent on the distribution of the disperse phase in the volume of the continuous phase with the required degree of uniformity. The results of the studies are presented in Table. 1.

Table 1

The dissolution rate of the components at the chosen temperature regime

Serial number	Temperature, °C	Number of revolutions of stirrer, sec ⁻¹	Duration of stirring to the absence of insoluble particles in solution, min.
1	20–22	50	20–30
2	20–22	100	15–20
3	20–22	150	10–15
4	20–22	200	10–15

When developing the technology of spray preparation, several preparation regimes were studied to obtain a solution that is free from undissolved particles-with different durations and mixing speeds. It was found that with a shorter mixing speed, a longer mixing time is required, and on the basis of the results obtained, an optimum dissolution time of 10–15 min was chosen which was fixed at a stirring speed of 150–200 sec⁻¹.

We also studied the quality indicators of the veterinary drug “Argocide-copper” after 12 months of storage. The results are shown in Table. 2.

As follows from **Table 1, 2**, the following technological modes of preparation of an anti-septic spray are considered to be the most optimal: dissolution temperature 20–22 °C, mixing time from 10–15 minutes, with stirring intensity from 150–200 sec⁻¹.

Table 2 shows the quality of the spray after 12 months. storage, which indicates that the spray produced by observing the selected optimal values of the dissolution process (temperature 20–220 °C, duration 10–15 min, stirring speed 150–200, sec⁻¹), retains the values of these indicators within the limits specified in the draft analytical documentation.

It can be argued that the selected technological mode of preparation of the spray based on citrate of silver and copper ensures the stability of the veterinary drug “Argocide-copper” during storage for 12 months.

The next stage of the research was the development of a technological process for obtaining a veterinary drug for external use. Preparation of a solution of an antimicrobial medicine based on active silver and copper ions was carried out in a reactor equipped with an anchor stirrer. Equipment in which the solution can be exposed to light must be covered. 50 litres of silver citrate and copper citrate solution were charged to the reactor and 20 litres of

purified water at a temperature of (20 ± 5) °C were added from the collector, then, sequentially: dexpanthenol was added to the reactor with stirring, and stirred for 10–15 minutes; polyquaternium – 10, mixed for 15–20 minutes.

Table 2

Quality indicators of the spray “Argocide-copper” after 12 months of storage.

Quality indicators according to regulatory documentation						
Serial number	Description (unclear liquid of a greenish-blue color)	Relative density, g/cm ³ (from 1.016 to 1.018)	pH (2.0–5.0)	Quantitative content, %		
				Ions of silver (not less than 0.0113)	Copper ions (not less than 0.0113)	Dexpanthenol (4.75–5.25)
1	+	1.016	2.15±0.01	0.0126±0.0002	0.0126±0.0002	5.10±0.01
2	+	1.017	2.13±0.03	0.0125±0.0001	0.0127±0.0001	5.09±0.02
3	+	1.016	2.14±0.03	0.0125±0.0002	0.0125±0.0002	5.09±0.01
4	+	1.016	2.15±0.03	0.0125± 0.0001	0.0125±0.0001	5.08±0.03

Note: + – matches; $P \pm 95\%$, $n=5$

Polysorbate 20 was placed in a separate vessel with a stirrer and a stirrer was turned on. With the stirrer on, lemon oil and juniper oil were added. Mixing was conducted until a homogeneous solution was obtained, at least 15 minutes. Then the contents of the collector, quantitatively transferred to a reactor with a solution of silver citrate, copper citrate, dexpanthenol and polyquaternium 10, were mixed until a homogeneous solution was obtained for at least 15 minutes. Then the solution volume in the reactor was adjusted to 100.0 L with water and stirred for 10–15 minutes. At the end of the preparation process, an average sample was taken to control the intermediate product according to the developed analytical normative document for the following parameters: pH of the solution; the quantitative content of silver and copper ions, dexpanthenol; appearance. The results are shown in **Table 3**.

After monitoring the intermediate product and obtaining positive results, the solution under pressure of inert gas (nitrogen) is transferred to the filters for filtration of the solution with charging by the “FILTRAK” filter paper of medium microporosity. It is necessary to control the brand of the filter, its integrity, the filtration regime (pressure 0.08–0.10 MPa). The solution after the filter enters the collection of the filtered solution. The filtered solution is monitored in terms of: description (hazy liquid of a greenish-blue color), coarse mechanical inclusions (absence), relative density (1.016–1.018 g/cm³). The results are shown in **Table 4**.

When the positive results of the intermediate control were received, the filtered solution entered the filling of the vials and their capping. The preparation of the primary packaging was carried out in accordance with the company’s document regulating the preparation of primary packaging materials.

Filling the vials with a solution and capping the caps of the spray was carried out on an automatic line for filling and capping the vials. After filling, the vials with the solution of the drug are moved to the capping zone, where the cap of the spray is screwed onto the neck. The intermediate product (bottles with solution) was monitored in terms of: the volume of the contents of the package (200 ml), the quality of the closure (no leakage of the solution, no deformation

of the vial and the spray cap). The sealed bottles are monitored on tables for viewing with the naked eye in a darkened room on a black and white background illuminated by a 100W electric lamp. Indicators for control: the presence of visible mechanical inclusions, the volume of filling (visually), the quality of capping. Vials with mechanical inclusions, incomplete dosage, as well as with vial defects and capping are discarded. The vials, which passed control, are given for labeling and packaging.

Table 3

The study of the quality indicators of the intermediate product in the preparation of the spray "Argocide-copper"

Serial number	Quality indicators according to regulatory documentation				
	Appearance (unclear liquid of a greenish-blue color)	pH of the solution (2.0–5.0)	The quantitative content, % silver ions (not less than 0.0113)	The quantitative content, % copper ions (not less than 0.0113)	The quantitative content, % dexpanthenol (4.75–5.25)
1	matches	2.17±0.01	0.0127±0.0002	0.0126±0.0002	5.03±0.03
2	matches	2.15±0.02	0.0126±0.0003	0.0127±0.0001	5.01±0.03
3	matches	2.14±0.03	0.0126±0.0003	0.0125±0.0003	5.01±0.03

Note: $P \pm 95\%$, $n=5$

Table 4

The study of the quality of the intermediate product (filtered solution) in the preparation of the spray "Argocide-copper"

Serial number	Quality indicators according to regulatory documentation					
	Description (unclear liquid of a greenish-blue color)	Relative density, g/cm ³ (from 1.016 to 1.018)	pH (2.0–5.0)	Quantitative content, %		
				Ions of silver (not less than 0.0113)	Copper ions (not less than 0.0113)	Dexpanthenol (4.75–5.25)
1	+	1.017	2.15±0.03	0.0127±0.0002	0.0126±0.0002	5.03±0.03
2	+	1.017	2.14±0.03	0.0126±0.0003	0.0127±0.0001	5.02±0.02
3	+	1.016	2.14±0.03	0.0127±0.0002	0.0125±0.0003	5.01±0.03

Note: + – matches; $P \pm 95\%$, $n=5$

Before being sent to the quarantine storage room, the TCD controller selects an average sample from the finished product series for a full analysis in accordance with normative analytical documentation. Before receiving the results of the analysis, the drug series is located in the quarantine storage room. The scheme of the technological process for the production of a veterinary drug is shown in Fig. 1.

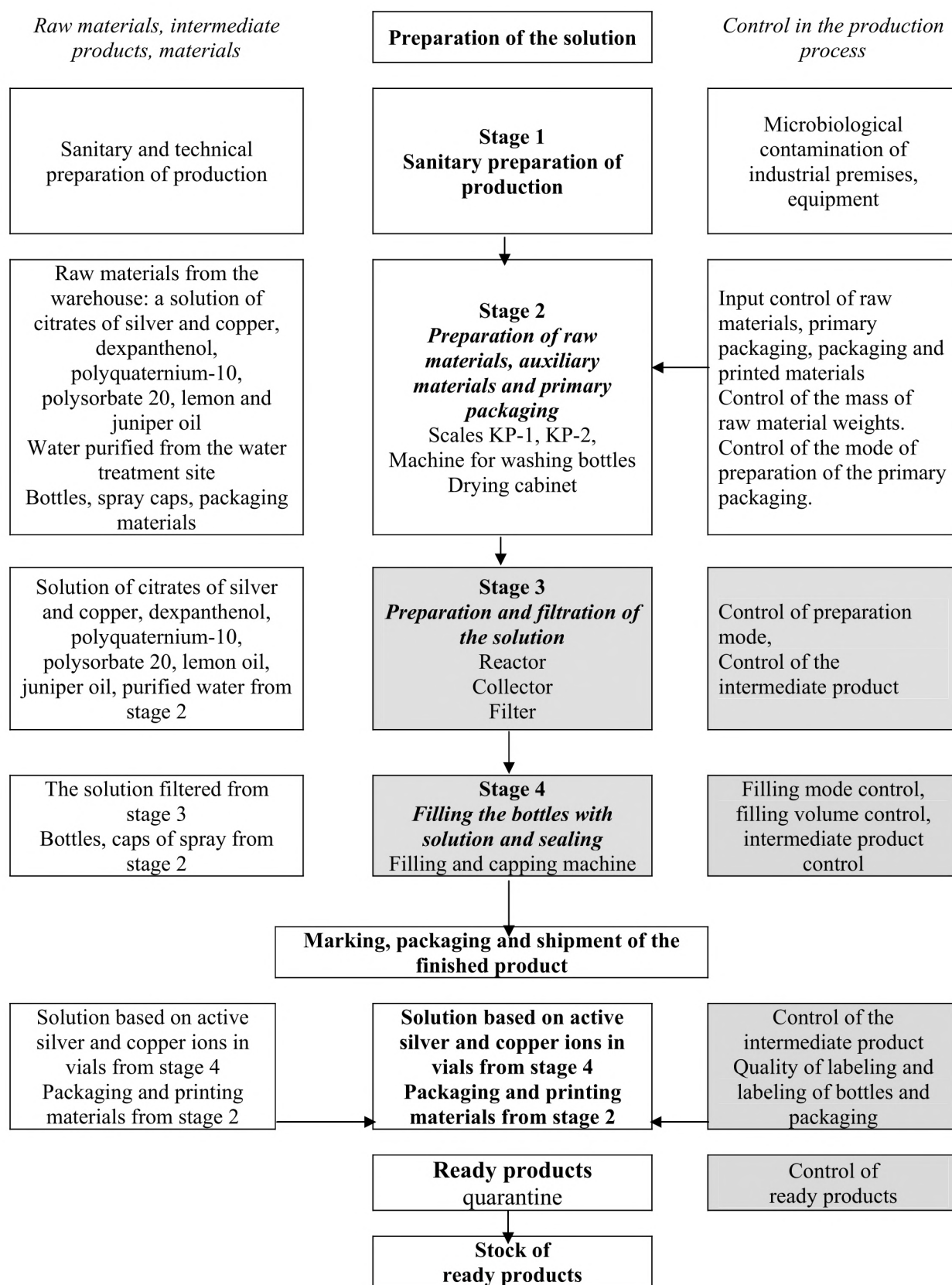


Fig. 1. Block diagram of the technological process of production of the drug “Argocide-copper” in the form of a spray: in gray are critical stages and critical control points in the production process

5. Discussion

It should be noted that the production of veterinary drugs in Ukraine in accordance with the requirements of good practices is at the implementation stage. The EU Commission adopted directives establishing the principles and rules of GMP for medicines and veterinary drugs. Directive 2003/94/EC refers to medicines for humans, and Directive 91/412/EC – medicines for veterinary use [19, 20]. The detailed requirements are in accordance with the principles of Directive 91/412/EEC set out in the “Guidelines for Basic Rules of Good Practice in the Production and Quality Control of Veterinary Preparations”, based on which the manufacturers of veterinary drugs in Ukraine will be inspected [21].

Medicines for veterinary use are made using materials and methods that ensure microbiological purity, prevent contamination of medicines and the growth of microorganisms. We tested the technology of the drug “Argocide-copper” under the conditions of the industrial production of veterinary preparations of LLC Brovafarm (Brovary, Ukraine), which allowed to develop and standardize it in normative and technological documentation (NTD). NTD consists of the technological regulations (it includes product data and technological instructions), packing instructions and corresponding protocols.

Production of the drug in the form of a spray based on active ions of silver and copper was carried out taking into account sanitary and hygienic requirements aimed at preventing microbial contamination of raw materials and finished products. For this purpose, the sanitary preparation of the production was carried out, which includes the operations of preparation of disinfectant solutions, preparation of ventilation air, production facilities, equipment and inventory, special clothing and personnel for work.

The process of production of the drug “Argocide-copper” was carried out in premises with a class of purity C, D, which is determined by the content of the maximum permissible mechanical particles and microorganisms in the air of the working zone. In the premises of cleanliness class C, the preparation, filtration, filling and sealing of the primary packaging, preparation of special clothes and personnel were carried out; in the purity class D, primary packaging (bottles, lids) was prepared, labeling and packaging of the vials into packs.

6. Conclusions

1. The effects of the preparation regimes and the order of administration of the components in the spray under the provisional name “Argocide-copper” on the stability of the preparation based on silver citrate and copper for veterinary medicine were studied.

2. It is found that the most optimal are the following technological modes of spray preparation: dissolution temperature 20–22 °C, mixing time from 10–15 minutes, with stirring intensity from 150–200 sec⁻¹. The chosen technological model of preparation of a spray based on citrate of silver and copper ensures the stability of the veterinary drug “Argocide-copper” during storage for 12 months.

3. The technology of obtaining a spray in the conditions of industrial production of veterinary preparations of LLC “Brovafarm” (Brovary, Ukraine) has also been developed. Critical stages and critical control points were determined during the production of a veterinary preparation of antiseptic action.

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