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ORIGINAL ARTICLE

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POSTOPERATIVE INFECTIONS AFTER GYNECOLOGICAL SURGERIES IN UKRAINE

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ABSTRACT

Aim: To determine the current prevalence of surgical site infections (SSIs) after gynecological surgeries and antimicrobial resistance of causing pathogens in Ukraine.

Materials and Methods: Multicenter prospective observational cohort study was conducted from January 2020 to December 2022 in nine hospitals from eight regions of Ukraine. Definitions of HAIs were adapted from the Centers for Disease Control and Prevention's National Healthcare Safety Network. Antibiotic susceptibility was done by the disc diffusion test as recommended by EUCAST.

Results: A total 12.2% (420/3450) patients who undergoing gynecological surgeries were found to have SSIs. The difference in SSI rates between the three subgroups by route of surgery was not statistically significant, being 12.0% for the abdominal group,11.1% for the vaginal group, and 12.5% for the combined group. The most common causing pathogens of SSIs was *Escherichia coli, Pseudomonas aeruginosa, Enterobacter spp., Streptococcus spp., and Klebsiella pneumoniae*. Many Gram-negative pathogens isolated from SSI cases were found to be multidrug resistant.

Conclusions: This study showed that SSIs remains the common complication after gynecological procedures in Ukraine. Best practices should be established and followed to reduce the risk of SSIs associated with gynecologic surgery. Optimizing the antibiotic prophylaxis and empirical antimicrobial therapy may reduce the burden of SSIs in gynecological surgeries, but prevention is the key element.

KEY WORDS: Surgical site infection, gynecological surgery, abdominal surgery, vaginal surgery, antibiotic prophylaxis, antimicrobial resistance

Introduction

Postoperative infection is the most commonly seen complication of surgery in gynecology. The most common infections after gynecological surgery include Endometritis, Parametritis, Vaginal cuff infection, Salpingitis, Oophoritis, Adnexa utery, Chorioamnionitis, and Pelvic abscess or cellulitis. Occurrence this infection is associated with the highest incidence of reoperation, the longest duration of hospitalization, and the greatest increase in cost of any postoperative gynecologic complication. According to literature, the prevalence of reproductive tract infection after gynecological surgery varies from country to country and ranges from 1.8% to 37.8% [1-3].

According to literature, postoperative infections after gynecological surgeries, and induced abortion is closely associated with a higher risk of infertility in women of reproductive age [4]. The results of study revealed high level the prevalence rate of SSI among infertile women of reproductive age in Ukraine is high. This applies to both primary and secondary infertility group women's [5, 6].

Antibiotic prophylaxis has been proved to decrease the infectious morbidity for vaginal procedures. Current international guidelines for the management of pelvic inflammatory disease recommend the prescription of antibiotics for prophylactic and treatment [1, 7, 8].

The use of antibiotic prophylaxis in gynecological surgery has greatly decreased though not completely eliminated this adverse outcome. In addition, the appointment of an inadequate starting therapy decreases the effectiveness of treatment. Irrational use of antibiotics is rampant. Antimicrobial resistance (AMR) is a global problem. One of the reasons for emergence of AMR is injudicious use of

antibiotics. Irrational use of antibiotics is rampant. Guidelines recommend administration of single dose of antibiotic for surgical antimicrobial prophylaxis for elective gynecological surgeries. There is no evidence to support prolonged use of antibiotics postoperatively in clean or clean-contaminated surgeries for prevention of post-operative infections. However, it is not usually adhered to in practice [9, 10]. Clinicians often overprescribe antibiotics presurgery and postsurgery sometimes for several days after surgery to overcome the fear of breach in asepsis during surgery and resultant SSI. Irrational antibiotic use might be harmful by altering the resident flora from susceptible to resistant strains.

Compliance with clinical practice guidelines is crucial for ensuring the appropriate and effective use of antibiotics. These guidelines offer recommendations for the selection, timing, and duration of antibiotic prophylaxis based on patient characteristics and the surgical procedure [11]. However, reports from Ukraine have shown that broader spectrum antibiotics, unnecessary combinations of antibiotics, suboptimal timing, and prolonged duration of surgical antibiotic prophylaxis are being used.

AIM

The aim this study to determine the current prevalence of postoperative infections after gynecological surgeries and antimicrobial resistance of causing pathogens in Ukraine.

MATERIALS AND METHODS

STUDY DESIGN, SETTINGS AND PARTICIPANTS

We performed a multicenter prospective observational cohort study was conducted from January 2020 to December 2022. Over study period, women who undergoing abdominal and vaginal surgery for benign gynecologic indications at nine hospitals from eight regions of Ukraine were included. Criteria for inclusion were those patients undergoing abdominal and vaginal procedures who were older than18 years of age and who had elective gynecologic surgery for nonmalignant pathology. Laparoscopic procedures were excluded unless combined with open surgery.

DEFINITION

An SSI was defined as an infection arising >48 h after surgery procedure and not present or incubating on admission, unless the patient had been discharged from hospital within a defined period. The criteria for specific type of SSI were adapted from the Centers for Disease Control and Prevention's (CDC) and National Healthcare Safety Network's (NHSN) case definitions. An incident SSI was defined by microbiologically confirmed CDC/NHSN HAI epidemiological case definitions. In addition, institution of antimicrobial treatment by a physician was not considered to be sufficient for diagnosis of an SSI because of widespread use of empiric antimicrobial therapy in Ukrainian hospitals.

MICROBIOLOGICAL METHODS

Species identification was performed with standard microbial methods. Antibiotic susceptibility testing of bacteria was determined by Kirby-Bauer disc diffusion test according to the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://eucast.org). An isolate is considered resistant to an antimicrobial agent when tested and interpreted as R in accordance with the EUCAST clinical breakpoint criteria used by the local laboratory. When combining results for antimicrobial agents representing an antimicrobial group, the outcome is based on the most resistant result.

DATA COLLECTION

Patients undergoing elective gynecological surgery and patients with SSI occurring within 30 days after the operation were involved in the study. Patients not willing to participate, patients below 18 years and above 60 years, patients who underwent re-exploration surgery, and patients who were operated on elsewhere and were referred for SSI or any other reason were not involved in the study. Patients were informed of the symptoms of SSI and advised to notify the observer right away after seeing the first SSI symptom for a month. The discharged patients were advised for ongoing follow-up care for a month in the outpatient department. Information regarding the postoperative course following discharge was obtained from the outpatient records and from records documenting postoperative follow-up by referring gynecologists or primary care physicians. Data were analyzed to document and classify all infectious morbidity and wound infection rates, and operative site infections were specifically documented for this analysis. In our study prophylactic antibiotic administration consisted of a second-or third-generation cephalosporin administered in a single preoperative dose.

ETHICS

The study was initiated after approval by the Institutional Ethics Committee of the Shupyk National Healthcare University of Ukraine (Kyiv, Ukraine). Patients selected for the study were required to sign an informed consent.

STATISTICAL ANALYSIS

All clinical and microbiological data results were entered in an Excel (Microsoft Corp., Redmond, WA, USA) database for statistical analysis. Continuous variables are expressed as mean and standard deviation (SD) or as median. Categorical variables are expressed as numbers and percentages. Data were analyzed to document and classify all infectious morbidity and wound infection rates, and operative site infections were specifically documented for this analysis. The factors used for subsequent analysis in each patient were the use of preoperative antibiotics, body mass index (BMI), presence of known diabetes mellitus, route of surgery, and history of smoking. We analyzed the association of these factors with infectious morbidity using Fischer's exact test. In our study significance was based on $P<0.05$.

RESULTS

POSTOPERATIVE INFECTIONS

A total in during study period based on our inclusion criteria, in total 3512 patients were eligible for analysis. Among the 3512 patients were 3450 for whom there were sufficiently detailed follow-up records to allow inclusion in this study

Table 1. Characteristics of surgical site infection (SSI) after gynecological surgery in Ukrainian hospitals (2020-2022)

Variables	All of Procedure n	SSI		P- value
		Yes N (%)	No N (%)	
Abdominal surgery				
Number of cases	2.250	270	1.890	0.079
Antibiotic prophylaxis	1.710	150 (5.55)	1.560 (82.5)	
Rehospitalization	150	120	30	
Repeat surgery	150	120	60	
Vaginal surgery				
Number of cases	810	90	720	0.49
Antibiotic prophylaxis	660	60 (66.7)	600 (83.3)	
Rehospitalization	60	60	0	
Repeat surgery	30	30	0	
Combined surgery				
Number of cases	480	60	420	0.083
Antibiotic prophylaxis	300	0 (0)	300 (71.4)	
Rehospitalization	60	30	30	
Repeat surgery	0	0	0	
Combined data				
Number of cases	3450	420	3030	0.012
Antibiotic prophylaxis	2670	210 (50.0)	2460 (81.2)	
Rehospitalization	270	210	60	
Repeat surgery	240	150	90	

(i.e., we were unable to obtain adequate postoperative records for the remainder). Among these, 2250 had abdominal surgery, 810 had vaginal surgery, and 480 had undergone combined vaginal and abdominal procedures. The average age of the patients was 41 years (range 24-68 years). The average height was 162.5 cm, and the average weight was 71.2 kg, with an average BMI of 27.31.

There were 420 patients with surgical site infection (SSI) among the 3450 total patients, an overall infection rate of 12.2%. The difference in SSI rates between the three subgroups by route of surgery was not statistically significant, being 12.0% (270/2250) for the abdominal group,11.1% (90/810) for the vaginal group, and 12.5% (60/480) for the combined group. Only of these 420 cases was detected in the initial hospital stay; the remaining 390 were identified only through examination of records of subsequent care after hospitalization. In this study fifty percent of the patients with infection required repeat hospitalization compared to only 1.98% patients without infection. 150 of the 420 patients with infection required a repeat surgical procedure directly related to the postoperative infection. The results of this are summarized in Tables 1 and 2.

ANTIBIOTIC PROPHYLAXIS

Among all subjects, 2670 patients received preoperative antibiotic prophylaxis. Of those who received antibiotic prophylaxis, 7.9% developed SSI (210/2670), whereas, among those who did not receive any antibiotic prophylaxis, 26.9% developed a postoperative infection. This difference was statistically significant (P=0.0123, RR=0.26, with a CI of 0.10-0.72) (Table 3).

LOGISTIC REGRESSION

We analyzed the data by logistic regression and found that after stepwise adjustment only antibiotic prophylaxis was a significant variable (P= 0.012, RR=0.28, with CI 0.11-0.72), whereas a history of diabetes mellitus, smoking, route of surgery, and BMI were not significant predictors of wound infection. These data are shown in Table 3.

ABDOMINAL SURGERY

There were nine wound or operative site infections among the 2250 patients with an exclusively abdominal route for surgery (12.5%). 150 patients with infections were readmitted, received intravenous antibiotics, and underwent wound incision and drain age of vaginal cuff abscess. Ten patients without operative site infection were readmitted for treatment of pneumonia, and 20 other patients in this group underwent repeat surgery for small bowel obstruction (un associated with the surgical wound). Among the 2250 patients, 1710 received antibiotic prophylaxis, and 450 did not receive antibiotic prophylaxis. Among

Table 2. Risk of surgical site infection (SSI) related to antibiotic prophylaxis in gynecological surgery in Ukrainian hospitals (2020-2022)

Variables	Number of women	Antibiotic prophylaxis given	No antibiotic prophylaxis	P-value
Abdominal surgery				
SSI	270	150	120	0.079
No SSI	1890	1560	330	
Total	2160	1710	450	
Vaginal surgery				
SSI	90	60	30	0.49
No SSI	720	600	120	
Total	810	660	150	
Combined Surgery				
SSI	60	0	60	0.083
No SSI	420	300	120	
Total	480	300	180	
Combined Data				
SSI	420	210	210	0.012
No SSI	3030	2460	570	
Total	3450	2670	780	

Table 3. Analysis of variables of Surgical Site Infection (SSI) by logistic regression

Variable	P- value
Antibiotic prophylaxis	0.012
Route of surgery	0.980
Body mass index	0.370
Smoking	0.330
Diabetes mellitus	0.293

Table 4. Bacterial pathogens (n=1,196) isolated from patients with Surgical Site Infection (SSI) after gynecological surgery in Ukrainian hospitals (2020-2022) ($P < 0.05$)

Microorganism	All isolates		Abdominal surgery		Vaginal surgery	
	n	%	n	%	n	%
Gram-positive cocci	**355**	**29.7**	**143**	**12.0**	**212**	**17.7**
Staphylococcus aureus	46	3.8	38	3.2	8	0.7
Staphylococcus epidermidis	18	1.5	7	0.6	11	0.9
Staphylococcus haemolyticus	14	1.2	6	0.5	8	0.7
Streptococcus spp.	96	8.0	28	2.3	68	5.7
Enterococcus faecalis	181	15.1	64	5.4	117	9.8
Gram-negative bacilli	**841**	**70.3**	**281**	**23.5**	**560**	**46.8**
Escherichia coli	412	34.4	137	11.5	275	23.0
Klebsiella pneumoniae	86	7.2	25	2.1	61	5.1
Klebsiella oxytoca	14	1.2	3	0.3	11	0.9
Enterobacter spp.	96	8.0	31	2.6	65	5.4
Proteus mirabilis	62	5.2	3	0.3	59	4.9
Serratia marcescens	28	2.3	13	1.1	15	1.3
Pseudomonas aeruginosa	102	8.5	46	3.8	56	4.7
Acinetibacter spp.	23	1.9	16	1.3	7	0.6
Stenotrophomonas maltophilia	18	1.5	7	0.6	11	0.9
Total	**1,196**	**100.0**	**424**	**35.5**	**772**	**64.5**

those receiving preoperative antibiotics, 8.9% developed an infection compared to 26.7% among those who did not. The difference in infection rates among women managed with or without antibiotic prophylaxis approached statistical significance (P=0.079, RR=0.32, with CI of 0.10-1.06). The average length of stay (LOS) was increased from 3.2 days to 5 days among those women who experienced wound or operative site infections.

VAGINAL SURGERY

In this study the observed rate of SSI was 11.1% (90/810). 60 patients in the infection group were rehospitalized and treated with intravenous antibiotics, and one of the two underwent transvaginal drainage of a cuff abscess in the ward. Of the 810 patients undergoing an exclusively vaginal procedure, 660 received preoperative antibiotics, and 150 did not. Among those who received preoperative antibiotics, the wound infection rate was 9.1% compared to 20% among those who did not receive prophylaxis (P=0.49, RR=0.48, with CI of 0.05-4.27). Infection increased the average LOS by 3.6 days in this group.

COMBINED SURGERY

The average rate of infection was 12.5% (60/480). 30 patients in each group were readmitted. In both groups, the reason for admission was unrelated to the surgical wound. Of note, none of the patients with infection received prophylaxis compared to 71.4% (300/420) of the patients without infection. There were no infections among those treated with antibiotic prophylaxis is compared to 33.33% among those who did not receive antibiotics (P=0.083). Again, an increase in average LOS from 2.3 to 4.5 days was seen with infection.

CAUSATIVE AGENTS AND ANTIMICROBIAL RESISTANCE

A total of 1,196 microorganisms were isolated from surgical wound secretion. The most frequent microorganism isolated was Escherichia coli (412 isolates), Pseudomonas aeruginosa (102 isolates), Enterobacter spp. (96 isolates), Streptococcus spp. (96 isolates) and Klebsiella pneumoniae (86 isolates). In this study of all SSIs cases 81.2% (341/420) were reported to be polymicrobial. Gram-negative bacteria predominated (70.3% of all organisms). The distribution of the microorganisms differed according to the after abdominal or after vaginal surgeries of the infection (Table 4).

Regarding the bacterial resistance, the main bacteria found were analyzed. In this study among the antimicrobial agents tested, the carbapenems (ertapenem) and piperacillin/tazobactam, and cefotaxim were the most consistently active in vitro against Enterobacteriales in both vaginal surgeries and after abdominal surgery infections. Proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriales was 17.5% and of methicillin-resistance in Staphylococcus aureus (MRSA) 9.7%. No vancomycin-resistance *Enterococcus faecalis* (VRE) strains were isolated. Vancomycin, teicoplanin, linezolid and fosfomycin, were the most consistently active *in vitro* in both vaginal surgery and abdominal surgeries infections, due to the strains of *E. faecalis*.

In the analysis for aminoglycosides against *E. coli*, the resistance was 8% for amikacin, 23% for gentamicin and 36% for tobramycin. When analyzing beta-lactams, the resistances were 11-81%, for carbapenems, 0-8% for furans and was 3%, specifically, for nitrofurantoin. Against *P. aeruginosa*, the carbapenems (meropenem, ertapenem, and imipenem), trimethroprim/sulfamethoxazole, amikacin and ticarcillin were the most active agents in vaginal surgery infections, while meropenem, ertapenem, and trimethroprim/sulfamethoxazole were the most active agents in abdominal surgery infection cases.

DISCUSSION

In this study we sought to determine the postoperative wound infection rate among patients undergoing elective gynecologic surgery and antimicrobial resistance of causing pathogens, and to determine the predictive value of various factors that contribute to infection in Ukraine. We further investigated the adequacy of hospital records in documenting infection rates as well as the timing of presentation of wound infections. The records of 3450 patients undergoing elective gynecologic surgery at nine regional women hospitals of Ukraine were reviewed. All patients were further subdivided based on route of surgery. We analyzed the importance of antibiotic prophylaxis, route of surgery, smoking, diabetes, and BMI. The overall SSI rate was 12.2% with no significant difference in the subgroups by route of surgery. Overall, antibiotic prophylaxis significantly decreased SSI rates, but the route of surgery, BMI, smoking, and diabetes were not significant predictors of infection. Only one case of infection was detected during the initial hospital stay (6.1%). Fifty percent of the patients with infection required readmission, and of these 35.7% required an additional surgical procedure. The average length of hospital stay was 2.4 days longer in patients with SSI.

According to literature, SSI is one of the most common complications in gynecologic surgery. Opening the lower genital tract exposes the pelvic operative site and the abdominal wound to vaginal flora in spite of preoperative vaginal preparation [12]. In our study of SSI rate after gynecological surgeries were 12.2%. Previous studies have shown that prevalence of SSI after gynecological surgeries in Ukraine was 4.6-38.8% [2, 3, 13-15].

Antibiotic resistance of microorganisms that cause infections of the urogenital tract is a clinically relevant problem in obstetrics and gynecology. Although the introduction of antibiotics allowed a marked increase in hope of life, it also increased alarmingly the bacterial resistance that threatens to impede this advance and poses significant risks to the safety of public health worldwide [16].

In the present study, the high level of resistance to multiple antibiotics is of great concern. The potential production of ESBLs detected is alarming. This condition represents an indication of seriously limited options for the treatment of patients infected with those microorganisms. Among Gram-negative bacteria, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* were the most frequently reported. This

finding is of particular concern, since these organisms are often involved in outbreaks that require the activation of an organizational response until the outbreak is under control. Due to methodological difficulties, resistance testing is rarely used for the management of these infections. Therefore, solid epidemiological data on resistance rates of most involved pathogens are scarce. Antibiotic resistance of several microorganisms appears to be increasing in various areas of the world. The presence of antibiotic resistance should therefore be considered in patients with an unfavorable course despite adequate antibiotic therapy. In light of the growing problem of antibiotic resistance and the large gaps in our knowledge in this particular area, research efforts in the field of anti-biotic resistance in gynecological infections should be markedly intensified.

The use of antimicrobial prophylaxis in obstetric and gynecologic surgeries varies considerably according to geographical areas and socioeconomic and cultural contexts [17]. The use of antibiotic prophylaxis has greatly decreased though not completely eliminated this adverse outcome. Antibiotic prophylaxis should be given to prevent SSI prior to gynecological surgery or procedures that enter the reproductive tract, ideally 60 minutes prior to skin incision. For procedures such as hysterectomy, antibiotic prophylaxis is clearly indicated, for others such as diagnostic laparoscopy, antibiotic prophylaxis is usually not required. For other procedures the evidence is less clear, and recommendations are based on expert agreement until further research evidence becomes available. Surgeons should consider each patient's individual requirements before prescribing the recommended antibiotic. The patient's risk factors for postoperative infection are predictable and need to be taken into account prior to any surgery, such as smoking status, diabetes, obesity, nutritional status, co-existent infection at a remote body site, vaginal colonisation with micro-organisms and immunodeficiency [8].

Postoperative infections observed after gynecologic surgical procedures are polymicrobial and require parenteral broad-spectrum antimicrobial therapy until the patient has cleared all symptoms of the infection. Broad-spectrum antibiotics should be initiated as soon as possible when diagnosis of postoperative infection is made; most patients will respond to treatment within 24 to 48 hours when appropriate antibiotics are selected. In addition, therapy should be tailored to the specific infection and patient response thereto.

The vagina contains more microorganisms than any other site in the body except the bowel. Uterine manipulation through the vagina, e.g., surgical termination of pregnancy, or operations that open the vagina, e.g., hysterectomy, will result in contamination of normally sterile sites by bacteria that are normally resident in the vagina. Whether these organisms become established and cause infection and inflammation depends on a mixture of surgical and host-related factors, including low socioeconomic status, poor nutrition, smoking, or preexisting medical conditions, such as impaired immunocompetence [8]. It is important to consider the likely source of pathogens in each type of surgery. Prior to any surgery, there is preparation with history, examination and appropriate investigations. Prior to gynecological surgery, screening for genital tract infection is not required; however, women with symptoms or risk factors should be tested and treated for sexually transmitted infections (chlamydia and gonorrhoea) and bacterial vaginosis. These have been associated with an increased risk of infection, endometritis with chlamydia and gonorrhoea following termination and vaginal cuff infection following hysterectomy with bacterial vaginosis [8].

Our study showed that postoperative infections after gynecologic surgical procedures can occur during the immediate postoperative period or after discharge from the hospital. As many as 50 per cent of these infections may occur after the patient is discharged from the hospital. Without question, the most appropriate means of objectively identifying infection rates, appropriate and inappropriate use of antimicrobials, and trends in morbidity is with an infection control program, usually consisting of an epidemiologist or specially trained nurses. Their contribution to appropriate patient care is significant. Implementing programs to reduce infections in the surgical field requires a collaborative approach involving clinicians, nurses, and staff.

STRENGTHS AND LIMITATIONS

The strengths of the study lie in the prospective nature, and application of CDS/NHSN methodology. It is well known that indicators of SSIs provided by surveillance activities require comparison with adequate reference data to stimulate further infection control actions and to enhance quality of care. Particular limitations in this study should be acknowledged when interpreting our findings. The limitations of this study include in conduct at a 33.3% region (8 from 24) in Ukraine. The results may not be representative of other regions of Ukraine with different distributions of SSI cases and antimicrobial resistance of responsible pathogens of infections after gynecological surgeries.

CONCLUSIONS

This study showed that SSIs remains the common complication after gynecological procedures in Ukraine. Most postoperative infections in gynecological surgery are treated empirically with antibiotics, making comprehensive resistance surveillance data essential to guide empiric regimens. Best practices should be established and followed to reduce the risk of SSIs associated with gynecologic surgery. Optimizing the antibiotic prophylaxis and empirical antimicrobial therapy may reduce the burden of SSIs in gynecological surgeries, but prevention is the key element.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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FEAR AND STIGMATIZATION IN THE GENERAL POPULATION DURING THE COVID-19 PANDEMIC

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ABSTRACT

Aim: The aim of the study was to investigate the feeling of fear and stigmatization in the general population, during the pandemic.

Materials and Methods: The study sample consisted of 1076 people attending a vaccination center in the Region of Argos, a prefecture of Peloponnese Region. The study was conducted from May 2021 to August 2021. In this study we used the Fear Investigation Scale(FCV-19S) and the COVID-19 Stigma scale.

Results: A total of 1076 participants took part in the study. Vaccinated participants for Sars Cov-2 had higher levels of fear ($U=117,569.00$, $p<0.001$) and individuals with unspecified employment status had a higher level of fear compared to civil servants, pensioners, unemployed, private employees and freelancers ($H = 17.225$, $p=0.004$). Higher levels of fear were also seen by those who reported that they were uncertain that the COVID-19 pandemic was likely to be treated soon compared to those who believed it would be treated soon and those who did not believed this ($H = 31.299$, $p<0.001$). Vaccinated citizens showed a higher stigmatization rate of health professionals. Professional uncertainty and unemployment shows that they are a factor for increased fear. Strong social and family ties also indicate the reduced presence of stigmatization.

Conclusions: An important imprint of the pandemic was the stigmatization of population's groups, such as health professionals, foreigners, as well as the infected by COVID-19. It is evident in society, that it was the fear of contracting the disease that contributed to stigmatization and isolation.

KEY WORDS: COVID-19, pandemic, fear stigma, vaccination

INTRODUCTION

The COVID-19 pandemic has adversely affected the lives of individuals, accompanied by biological and behavioral, psychological, economic and social changes [1]. Dealing with the pandemic constitutes the necessary condition which, in addition to the actions of the state, strongly highlights the need for individual responsibility and the creation of an individual framework through which the individual is called upon to manage the disease 2].

The fear of the unknown disease, such as the COVID-19 pandemic, has been studied a lot in recent years [3,4]. It seems that this fear can lead to an increased tension, anxiety, depression and burden of the psycho-emotional spectrum of the individual and societies [5]. Furthermore, the fear of contracting the disease can be a key factor in causing excessive or even extreme manifestations and reactions from individuals, such as competition, overprotection, stigmatization and isolation [6].

According to WHO, social stigma in the context of health is the negative relationship between persons, or a group of people, who have certain characteristics or suffer from a specific disease. In a pandemic, this can result in individuals being discriminated against through stereotypes that are created, resulting in them being treated differently, depending on their relations hip with the disease [7]. Within a society can undermine social cohesion and lead to the isolation of individuals or even groups, while, regarding the relationship with the spread of the virus, this can worsen [8].

The COVID-19 pandemic has brought to surface stigmatization, the focus of which varies across different phases of the pandemic [9]. Discriminatory attitudes may concern people with specific ethnic or professional characteristics, such as health professionals, because of their frequent contact with the virus. The stigmatization of health professionals is a phenomenon that emerges during situations such as pandemics [10].

AIM

The purpose of the study was to investigate the feeling of fear and stigmatization in the general population, during the pandemic.

MATERIALS AND METHODS

STUDY'S SAMPLE

The study sample consisted of 1076 people attending a vaccination center in the Region of Argolis, and specifically the vaccination center of the Argos Nursing Unit. The study

was conducted from May 2021 to August of the same year, a period when the pandemic and vaccination of the general population were in progress.

RESEARCH TOOLS

The study instrument included demographic characteristics and two weighted scales, the Fear Investigation Scale(FCV-19S) [11] which has been translated and validated in Greek population [12] as well as in Greek health professionals[13], and the COVID-19 Stigma scale (COVID-19 stigma), [14] which has also been validated in the Greek language [15].

The Fear of COVID-19 Scale is a self-reporting tool consisting of 7 items that assess fear of COVID-19.The FCV-19S comprises seven items, each with a five-point Likert scale of answers ranging from strongly disagree (1) to strongly agree (5). Total score of the FCV-19S ranges from 7 to 35 with higher values indicating greater fear. Cronbach's alpha for fear of COVID-19 scale was 0.79 indicating good internal consistency.

The COVID-19 stigma questionnaire consists of two sections. The first section includes general population's demographic characteristics such as gender, age and the second section includes questions regarding the stigma associated with COVID-19 infection, presenting a dichotomous response set of answers (yes or no).Saridi et al. (2022) translated and validated this questionnaire using a sample of 35 people from the Greek general population. In their study they reported high levels of face and content validity as well as reliability and the authors concluded that their findings suggest that this questionnaire may be used to quantify stigma discrimination toward the general population.

ETHICAL CONSIDERATIONS

Throughout the study, the anonymity and confidentiality of the participants were maintained, while participation in the study was completely voluntary. The approval of the research protocol was given by the scientific council of the General Hospital where the research was conducted (Prot. No. 5612,11.05.21). The participation of the experts and people was anonymous and voluntary.

STATISTICAL ANALYSIS

Absolute (n) and relatives frequencies (%) were used to describe sociodemographic variables. To compare two or more groups, student's t-test, Mann-Whitney and Kruskal-Wallis test were used, according to whether data were normally distributed or not. Levels of significance are two-tailed and statistical significance was set at 0.05. The statistical program SPSS (Statistical Package for the Social Sciences for Windows), version25.0was employed for all analyses.

RESULTS

A total of 1076 participants took part in the study: 662 females (61.5%) and 414 males (38.5%). Most of the participants 427 (39.7%) belonged to the age group of 41-59, and the majority was married 663 (61.6%). A total of 197 (18.3%) were unemployed and only 183 (17%) continued to work in their workplace during the period of restrictive measures. At the time of the study only 38 (3.5%) have already been sick by Sars Cov-2 and the majority 679 (63.1%) have been vaccinated. 334 (31.1%) of the participants have no or moderate confidence in the health system for the treatment of COVID-19 and the rest trust it quite well. At least 855 (70.2%) think that the pandemic of the new Covid-19 coronavirus will be addressed soon (Table 1).

Vaccinated participants for Sars Cov-2 had higher levels of fear (U=117,569.00, p<0.001) but also higher levels of stigma discrimination toward health workers (HW) (U=123,215.00, p=0.008) suggesting that the fear of Covid-19 was a factor in both vaccination and targeting population groups like HWs.

Individuals with unspecified employment status had a higher level of fear compared to civil servants, pensioners, unemployed, private employees and freelancers (H = 17.225, p=0.004). If this was combined with a change in the relationship of existing work, temporarily suspended people had higher levels of fear compared to those who worked with presence in their workplace, those were working remotely and those who were fired or quitted working, during Covid-19 pandemic (H=9.350, p=0.027), we may conclude that job insecurity results in higher level of fear of Covid-19.

In addition, those reported that they could turn to their roommate in case of emergency, had higher levels of stigma toward health workers than those responded that their children, friends, partner or spouse would help them (H=13.271, p=0.021). This probably means that structured relationships and strong bonds with people can reduce stigma discrimination.

In different subgroups, women compared to men, those who had suffered from some chronic physical disease and those who did not exercise more than 3 hours per week had higher levels of fear. Concerning the stigma toward health workers, it was higher in men and those who had more than 4 children (Table 2).

FEAR OF COVID-19 AND STIGMA DISCRIMINATION TOWARD HEALTH WORKERS REGARDING LEVEL OF EDUCATION

In terms of level of education, we found that elementary school graduates had higher levels of fear compared to postgraduate students, high school graduates, university graduates, holders of postgraduate degree and PhD holders (H=17.369, p=0.003). This correlation is reversed when we refer to stigma discrimination toward health workers. Hence, PhD holders had higher levels of stigma compared to the graduates of elementary, high-school, lyceum, university, and holders of postgraduate degree (H=31.430, p<0.001) (Figure 1).

FEAR OF COVID-19 REGARDING CONFIDENCE IN HEALTH SYSTEM/STAFF AND DURATION OF THE PANDEMIC

Those reported having sufficient confidence in the health system had higher levels of fear than those who had very, excellent, moderate or no confidence (H = 54.433, p<0.001). Respectively, those who answered that they have enough confidence in medical and nursing staff to deal with the new disease, had higher levels of fear compared to those who had absolute, very, moderate and no confidence (H = 40.505, p<0.001). Higher levels of fear were also seen

Table 1. Sample demographics

	Frequency	No.	%
Gender	Male	414	38.5
	Female	662	61.5
Marital status	Married	663	61.6
	Single	275	25.6
	Divorced	76	7.1
	Widowed	27	2.5
	In cohabitation	35	3.3
Age group	18-29	196	18.2
	30-40	313	29.1
	41-59	427	39.7
	60-70	87	8.1
	>71	53	4.9
Employment status	Unemployed	197	18.3
	Privateemployee	263	24.4
	Stateemployee	223	20.7
	Freelance	202	18.8
	Retired	119	11.1
	Other	72	6.7
Work during the period of restrictive measures	Suspended	370	34.4
	Remotely working	403	37.5
	Yes, normally in my workplace	183	17.0
	I stoppedworking	120	11.2
Are you sick with Covid-19	No	1038	96.5
	Yes	38	3.5
Have you been vaccinated for Covid-19	No	397	36.9
	Yes	679	63.1
Do you trust the health care system for treating Covid-19	Notatall	76	7.1
	Moderate	258	24.0
	Enough	340	31.6
	Very	275	25.6
	Excellent	127	11.8
Do you think the pandemic of the new Covid-19 coronavirus will be addressed soon	No	321	29.8
	Yes	327	30.4
	Maybe	428	39.8

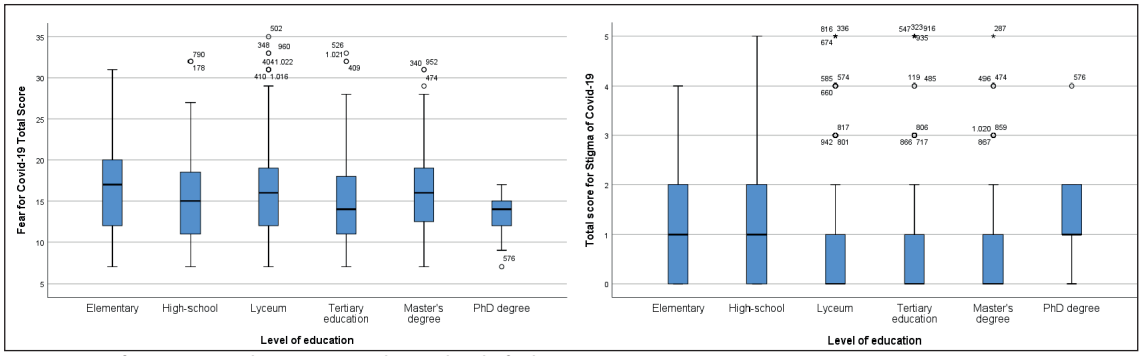

Fig. 1. Fear of COVID-19 and stigma, according to level of education

Table 2. Differences between subgroups

	Subgroups	Value
Fear of Covid-19		
	Women vs Men	U=103,937, p<0.001
	Vaccination for Sars Cov-2 /Yes *vs* No	U=117,569, p<0.001
	Chronic physical disease /Yes *vs* No	U=83,014, p=0.013
	Physical activity over 3hs per week/No *vs* Yes	U=127,754, p=0.013
	Unspecified employment status *vs* Civil servants-retired-private employees	U=17,225, p=0.004
	Temporarily suspended people *vs* working in presence, remotely-stopped working	H=9.350, p=0.027
Stigma discrimination		
	Men vs women	U=127,818, p=0.039
	Vaccination for Sars Cov-2 /Yes *vs* No	U=123,215, p=0.008
	Turn to roommate	H=13.271, p=0.021
	More than 4 children	H=24.994, p<0.001

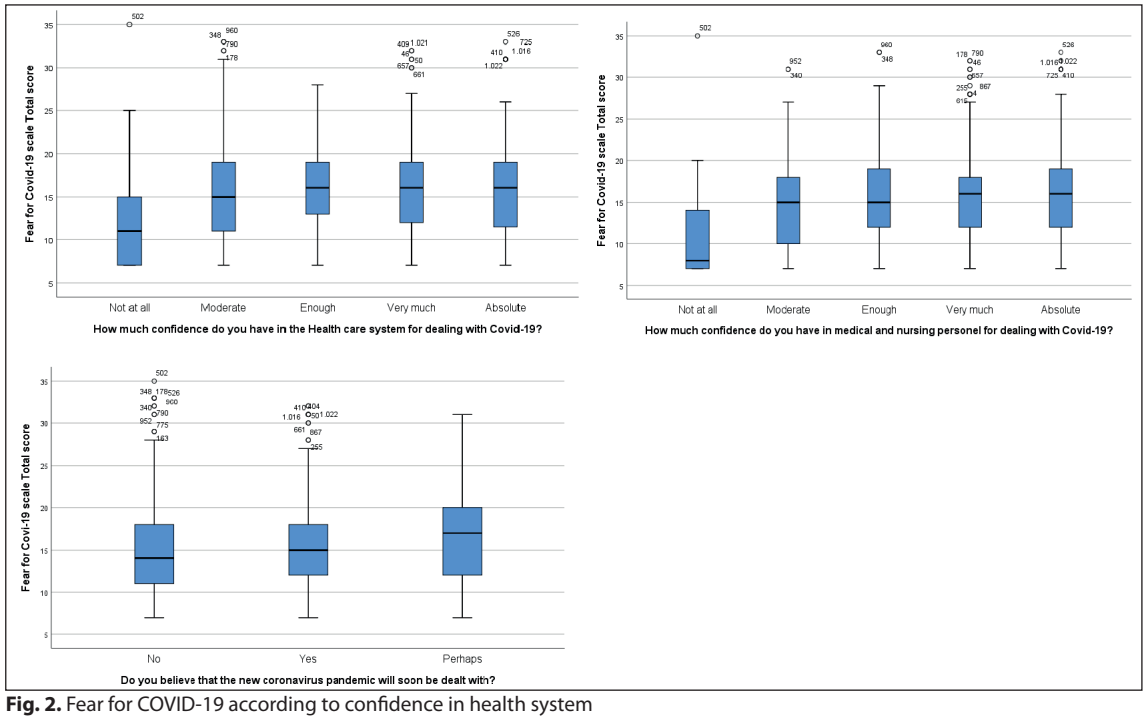

Fig. 2. Fear for COVID-19 according to confidence in health system

by those who reported that they were uncertain that the COVID-19 pandemic was likely to be treated soon compared to those who believed it would be treated soon and those who did not believed this (H = 31.299, p<0.001)(Figure 2). A clear view on these questions (e.g. yes / no, not at all/ /much) seems to reduce fear regardless of its content.

Participants of middle and higher age groups exhibited, comparatively, greater fear for Covid-19 (x^2=27.908, p=0.006) and stigma (x^2=14.031, p=0.007) (Figure 3).

Participants who reported that in case of need they turn to their partner for help, had higher anxiety levels (x^2=32.131, p=0.006) (Figure 4).

Tertiary education graduates and people residing in villages exhibited, comparatively lower anxiety levels (x^2=45.425, p<0.001 andx^2=15.479, p=0.017, respectively) (Figure 5). Unmarried and co-habitating participants showed, comparatively, higher stigmatization levels (x^2=13.268, p=0.004) (Figure 6).

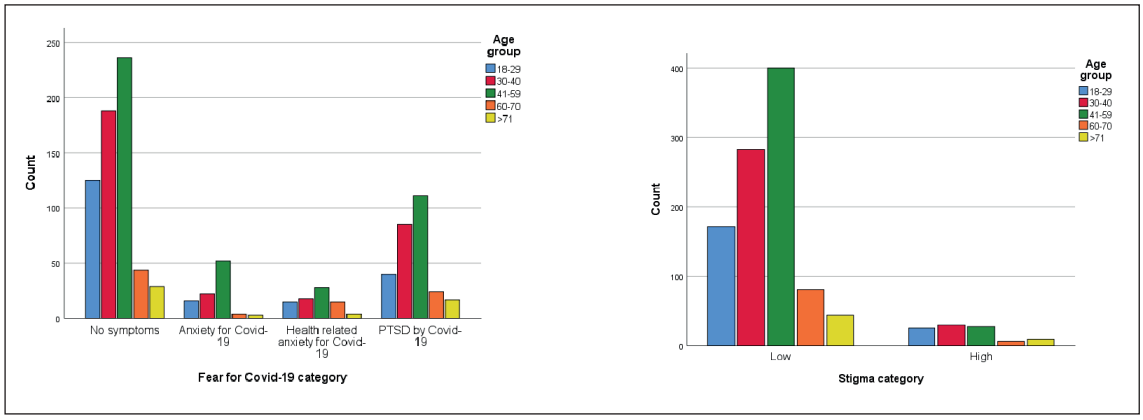

Fig. 3. Fear for COVID-19 category and stigma category according to age group

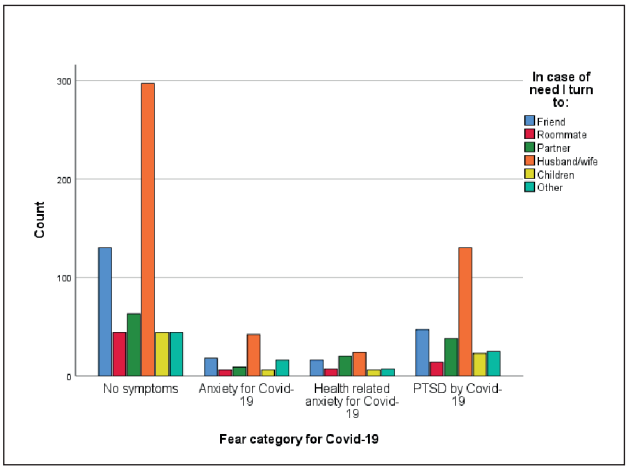

Fig. 4. Fear for COVID-19 category according to the person people turn to in cases of need

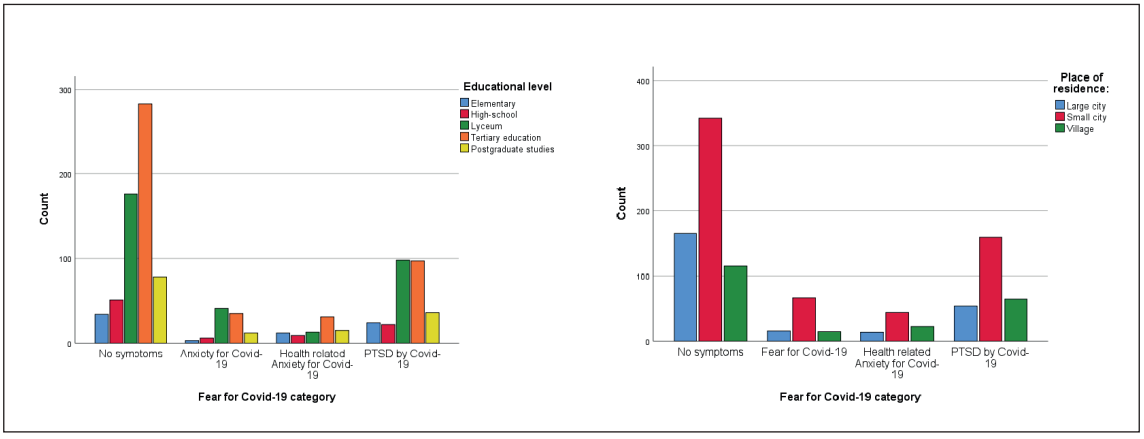

Fig. 5. Fear for COVID-19 category educational level and place of residence

DISCUSSION

The COVID-19 pandemic resulted in an important lesson for the whole planet which, in addition to the medical approach to the disease and the effort to deal with it, also experienced the fear of the unknown and invisible enemy that triggered the outburst of many emotions.

The purpose of this study was to investigate the feeling of fear and stigmatization in the general population, during the pandemic. The study was conducted from May 2021 to August of the same year, a time period when the pandemic and vaccination of the general population were in progress.

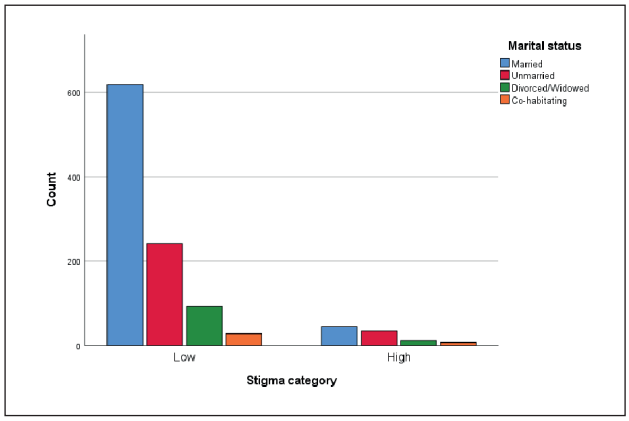

Fig. 6. Stigma category according to marital status

The measurement of fear of the coronavirus through the FCV-19S scale [11]. Was combined with the measurement of the stigmatization of health professionals throughout the course of the pandemic with the COVID-19 stigma scale [14].

The majority of the present study's sample belonged to the age group 41-59 years and this occurred mainly to the fact that during that time, this age group were vaccinated, according to the national vaccination program. Women and people suffering from chronic diseases exhibited higher levels of fear, while, the educational level and living in rural areas seemed to lower fear levels.

Although the Covid-19 pandemic is not over yet, several studies have tried to outline its effects on the people's psycho-emotional sphere and especially the fear that can be released by it. A systematic review pooled the results of studies on the impact of fear of the possibility of catching the disease [16]. A study in India showed high levels of fear 17]. While studies conducted in the areas of Bosnia and Hercegovina demonstrated lower levels [18]. Women seem to have higher levels of fear and this finding is congruent with other similar studies, [19-21] although, in contrast with study where male gender was connected with higher levels of fear compared to females [22].

At the same time, low educational level seems to play an important role in the increased incidence of fear of catching this disease [17,22].

The increase of fear at older ages was significant in our study. Internationally, there are heterogeneous results regarding the impact of fear on older people. A study from India shows that people over 60 are at lower risk of developing fear than younger people, while a study from Germany shows that older people have increased feelings of fear, which may be due to the culture of these population groups 23].

An independent finding was the level of fear in relation to citizens' trust in the health system, given that fear appears to be higher in people who reported trust in the health system and in the provision of services by health professionals. The uncertainty of dealing with the pandemic in such a short period of time was another factor connected with increased fear. In relative studies, it appears that there is a positive correlation between fear, risk perception and trust in the health system, factors that strengthen prevention measures [24,25].

As for stigmatization, it seems to be more common in men, in people who reported having a mental illness, in those who had an incident of illness, in vaccinated, people who live alone and retirees, those who had a high educational level, but also in participants who reported that they did not trust the health system.

International studies reflect the stigmatization of people who contracted COVID-19 while, their families experienced social stigma as well. It seems that fear and lack of knowledge about the disease are the main factors of stigmatization, while stigma has been associated with negative effects, especially on mental health, and is an important public health problem especially in times of pandemic which requires complex and difficult management [26,27].

An important finding in our study is the fact that vaccinated citizens showed a higher stigmatization rate of health professionals. Professional uncertainty and unemployment shows that they are a factor for increased fear. Strong social and family ties also indicate the reduced presence of stigmatization.

Several incidents of discrimination against patients with COVID-19, their environment and health workers appeared especially during the first year of the pandemic [28, 29, 30]. Social stigma of people suffering with COVID-19 is a global problem and can be compounded by race, occupation, social status, religious identity and vaccination status [31, 32].

CONCLUSIONS

Fear has been the dominant social emotion since the beginning of the phenomenon, but, at the same time, it still decisively directs every individual decision that affects the evolution and course of the pandemic. The fear of contracting the disease in the initial stages of the pandemic was great, especially during the first year of the pandemic, until someone could be vaccinated and thus may be considered that their life is more protected. The fear of the disease has led most of the Western world to vaccination, while, in the same way, the fear of being vaccinated has led a significant portion of society to concerns and doubt.

The impact of the COVID-19 pandemic on people's mental health, their quality of life and the increase in stress and loneliness, is a result that has been described in several studies. The burden on mental health can be the result of worry about the possibility of infection or the anxiety caused by confinement and other restrictive measures, self-isolation and social distancing, but also the loss of employment, income or education.

An important imprint of the pandemic was the stigmatization of population's groups, such as health professionals, foreigners, as well as the infected by COVID-19. It is evident in society, that it was the fear of contracting the disease that contributed to stigmatization and isolation. The

current COVID-19 outbreak has provoked social stigma and discriminatory behaviors against people of certain ethnic backgrounds as well as anyone perceived to have been in contact with the virus (WHO, 2020a).

Global health related phenomena such as the COVID-19 pandemic, are bound to affect the quality of life of numerous individuals and can lead to wide spread stigmatization, fear and anxiety. Therefore, it is essential for every government to employ programs for protecting and providing psychological support to vulnerable people or groups, as well as provide valid scientific suggestions and information to all members of society.

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PULMONARY DISEASE DIAGNOSTIC AND REHABILITATION TREATMENT FOR PATIENTS WITH OVERLAP ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASES

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Abstract

Aim: To analyze the effectiveness of rehabilitation treatment in the conditions of halo aerosol therapy and when using different groups of fixed-dose aerosol inhalers in patients with asthma, chronic obstructive pulmonary diseases and asthma-chronic obstructive pulmonary diseases overlap.

Materials and Methods: A total 112 patients with asthma, chronic obstructive pulmonary diseases and asthma-chronic obstructive pulmonary diseases overlap. All patients carefully collected allergic history, anamnesis of life, and disease. The study includes assessment of resting anthropometric data, physical activity habits, blood pressure, structure and quality of nutrition, family and economic data.

Result: After a course of rehabilitation treatment, lung ventilation improved in all groups of patients compared to controls. The dynamics of FER indicators after the rehabilitation treatment had some differences for each group of patients in relation to the control groups. If in all A groups (control) the increase in FER indicators was insignificant, then in all B groups of patients it was significant, and differed from 14,6% up to 36%.

Conclusions: Thus, a differentiated approach to the use of fixed-dose aerosol inhalers increases the effectiveness of rehabilitation treatment under conditions of halo aerosol therapy in patients with various genesis of bronchi-obstructive syndrome.

KEY WORDS: asthma, chronic obstructive lung disease, asthma – chronic obstructive lung disease overlap, bronchodilation test, bronchial obstruction.

INTRODUCTION

In times of globalization, rapid urbanization, and aging of the population, chronic diseases of non-infectious origin are becoming the most common. There are four types of such diseases: cardiovascular, oncological, chronic respiratory (asthma (A) and chronic obstructive pulmonary disease (COPD)) and diabetes. This «epidemic» affects all countries of the world without exception, and its socio-economic consequences are becoming more and more threatening [1].

In 2012, among 56 million deaths in the world, 38 million (68%) were caused by non-communicable diseases. 4 million people die annually from a and COPD. According to forecasts of the World Health Organization, by 2030, losses from chronic pathology will amount to 52 million people [1, 2]. In Ukraine, the situation is even more difficult, if in the world mortality from chronic pathology is 539 cases per 100,000 population, then in our country – 749 cases [2].

In Ukraine, more than 3 million residents may suffer from COPD, but only one in ten has a diagnosis [3, 4]. This problem has a global character, since even in the USA more than 12.5 million residents have a diagnosis of COPD, and another 24 million have a lung function disorder, but without confirmation of the diagnosis [2, 3, 5, 6]. According to the latest projections, COPD from the sixth cause of death in 1990 will take the fourth place in 2030 [4].

As for asthma, the prevalence of the disease in Ukraine, according to official statistics, is 488.8 patients per 100,000 adult population. Every year, asthma is diagnosed for the first time in about 8,000 people. These figures are significantly underestimated, since the diagnosis is established in every eighth case [7]. Over the past 25 years, there has been a 69.3% increase in the prevalence and a 22.9% increase in the incidence of asthma in Ukraine [8, 9]. Diagnosis of asthma is a difficult task, especially in the case of a non-classic clinical course of asthma and when symptoms of asthma and COPD are combined [10, 11]. Statistics do not reflect the true picture of the spread of asthma in Ukraine and testify to its underdiagnosis. Screening – survey made it possible to detect 7.5% of asthma patients in the world [12-15].

BA and COPD have certain common features for both diseases – chronic inflammation and impaired patency (obstruction) of the respiratory tract, but they differ significantly in pathogenesis, pathophysiology, diagnostic features, clinical course, pharmacotherapy schemes, consequences for quality of life, as well as the scale of impact on social – the economic situation of individuals

and society as a whole [2, 3, 7, 16]. The possibility of a concomitant course of asthma and COPD is from 10 to 25% for patients with asthma [7].

Recently, scientists often talk about the heterogeneity of asthma. The reflection of the heterogeneous nature of asthma is emphasized in the GINA guidelines: in the new definition of the disease, the selection of phenotypes and endotypes and the consideration of their features in the selection of effective treatment regimens [8, 9].

A new term was introduced into clinical practice in 2014, which means a combination of asthma and COPD – ACOS (asthma – COPD overlap syndrome). ACOS is referred to by GINA 2014, which was created by the joint efforts of two committees, GINA and GOLD [16-18]. On the basis of this document, a separate document «Diagnosis of diseases with chronic airflow limitation: asthma, COPD and ACOS» was developed. ACO is characterized by persistent restriction of airway patency with certain features that are characteristic of asthma, as well as with certain features that are characteristic of COPD [16]. Pulmonologists encounter this syndrome from 2 to 55% according to various epidemiological studies [19, 20].

At the heart of the pathogenetic mechanisms of the development of asthma and COPD is the bronchi-obstructive syndrome – a violation of the patency of the bronchi, which is accompanied by shortness of breath, remote and auscultatory dry wheezing on expiration, involvement of auxiliary respiratory muscles in the act of breathing [2-4, 7, 12, 16] . In asthma, the bronchi-obstructive syndrome is more dynamic. It develops as a result of variable bronchospasm of the bronchioles, which passes either independently or under the influence of medical drugs [7, 10, 11]. COPD is characterized by a permanent restriction of air permeability, which usually progresses and forms static hyperinflation of the lungs, shifts the pressure balance points on exhalation and expiratory collapse of the bronchi. All this leads to the formation of an air trap, and because of this – to emphysema of the lungs [5, 6].

ACO combines both mechanisms of bronchial obstruction and this allows us to consider it as a separate phenotype of asthma – COPD [17, 20].

The tone of the smooth muscles of the bronchi can be influenced through the stimulation of β2-adrenoceptor and blockade of M3-cholinergic receptors. M-cholinergic receptors are mainly concentrated in large and medium bronchi, while β2-adrenoceptors – in the middle and upper bronchi. In addition, M-cholinergic receptors retain sensitivity in all age groups and are resistant to tachyphylaxis, while sensitivity to β2-adrenoreceptors decreases with age with the possible development of tachyphylaxis [16, 17, 19].

The issue of treatment of such patients has been repeatedly raised in the scientific literature with different approaches to this problem. And only the acceptance of the term «overlap», its definition, as well as recommendations for the treatment of patients with ACO, which was the result of a joint project of GINA and GOLD, outlined the ways to solve this issue [20].

AIM

The aim was to analyze the effectiveness of rehabilitation treatment in the conditions of halo aerosol therapy and when using different groups of fixed-dose aerosol inhalers in patients with asthma, chronic obstructive pulmonary disease and asthma – chronic obstructive pulmonary disease overlap.

MATERIALS AND METHODS

A total of 112 patients with asthma, COPD and ACO were examined on the basis of the SDC «Rehabilitation» of the Ministry of Health of Ukraine in Uzhhorod which has an accreditation. All patients carefully collected allergic history, anamnesis of life, and disease. The study includes assessment of resting anthropometric data, physical activity habits, blood pressure, structure and quality of nutrition, family and economic data. 112 patients with asthma, COPD, and ACO who underwent a course of rehabilitation treatment under conditions of halo aerosol therapy (HAT) were examined. Of them, 50 patients with asthma were in the I group, 33 patients with COPD were in the II group, and 29 patients with ACO were in the III group. Each group of patients was divided into two subgroups (A and B). A – a subgroup of patients received rehabilitation treatment in the form of stay in the conditions of GAT. 26 patients with asthma were included in I-A, 16 patients with COPD in II-A, and 15 patients with ASO in III-A. I-B – subgroup for before the GAT session received dosed aerosol inhalation (DAI) of salbutamol (24 patients with asthma) [21], II-B – DAI of atrovent (17 patients with COPD) [22] and III-B – DAI of combivent (14 patients with ACO) [23]. Before the 21-day rehabilitation course of treatment, and after its completion, all patients underwent a functional study of the ventilation capacity of the lungs using the Pulmovent-2 computer spirograph. All patients underwent spirometry according to the standards of the American Thoracic Society and the European Respiratory Society [24, 25]. Forced expiratory volume (FEV1) were evaluated1, Forced vital capacity (FVC) and ratio of FEV1/FVC. The presence of bronchial obstruction was ascertained during FEV1/FVC<0.7. The bronchodilation test (BDT) was performed in accordance with the recommendations for standardization of spirometry [25, 27] using inhalation of 400 ml of salbutamol through a spacer and repeated examination after 15 minutes.

When saving FEV1/FVC<0.7, incompletely reversible obstruction was noted.

If the indicator is >0.7, The obstruction was marked as reverse. A pronounced response to a bronchodilator was established in the presence of an increase in FEV1 of ≥12% and≥200 ml; otherwise, they talked about the lack of a clear response to the bronchodilator. According to the results of the BDT, the diagnosis was revised [26, 27].

RESULTS

A survey of 112 patients with asthma, COPD and ACO was conducted according to the Helsinki Declaration on Biometric Research Involving Human Subject.

The average age in the (I) group of patients with asthma was 34.9±0.77 years, in the (II) group of patients with COPD

– 58.2±0.96 years, and in the (III) group of patients with ACO – 43.7±0.65 years.

In terms of gender composition, women predominated in groups I and III, and men in group II.

The onset of asthma in the group of patients with ACO (III) from 29 patients in 12 cases was formed in the same way as in the group of patients with asthma (I) at the age of 11.6±1.96 years. In the second group of 17 patients, symptoms of asthma were formed at the adult age of 38.4±0.87 years against the background of existing COPD.

When studying the hereditary anamnesis of patients with asthma, COPD, and ACO, various manifestations of allergies were found in the family in 48 (42.9%) cases.

Analyzing the anamnestic data, it was found that asthma, pollinosis, atopy, and other allergic conditions were primary in the development of ACO in 20.7% (6) of cases. In 79.3% (23) cases, COPD was the primary factor in the development of ACO.

Seasonality of exacerbations in the autumn-winter period with metrology to wet, windy, cold weather was characteristic for patients of III group. The majority of patients in this group pointed to a cold, contact with chemicals, and smoking as the cause of the onset of the disease. In 65.5% (19) of these patients, sensitization to household allergens was observed, including 27.6% (8) to tobacco smoke.

Inflammation of the respiratory tract was determined by general clinical and immunological methods. Thus, IgE in the group of asthma patients was 18.6±1.28 um. unit, in the group of patients with COPD – 4.7±1.08, in the group of patients with ACO – 9.4±1.14. The level of eosinophils in peripheral blood was 9.4±0.56% in group I, 3.6±0.38% in group II, and 5.1±0.64% in group III.

Performing BDT is an integral component of diagnostic measures and significantly improves the quality of diagnosis of COPD and asthma. The quality of diagnostics in general practice is low. Carrying out adequate spirometry with BDT made it possible to rule out COPD in 50% of patients with a diagnosis established by the primary care unit. Among them, asthma occurred at 19.7%. In 30.5% of patients, the diagnosis of COPD required clarification. These patients were included in the III group of patients with ACO.

Among patients with confirmed COPD, incompletely reversible obstruction with a significant response to a bronchodilator occurred in 7.6% of people.

When admitted to rehabilitation treatment, 90% of patients were found to have a variety of violations of bronchial patency and pulmonary ventilation. Ventilatory insufficiency according to the obstructive type was found in 76.2% of patients, according to the mixed – obstructive-restrictive type, it was established in 20.6% of the examined, and only 3.2% of patients had a restrictive type of violations. According to the gradations of violations of bronchial patency, slight changes in bronchial patency were found in 7.9% of patients, moderate changes in 55.6% of the examined patients, significant changes in the function of external respiration (FER) were detected in 25.3% of patients, sharp generalized violations of the function of external breathing, accompanied by a decrease in FVC was observed in 11.1% of patients. Studies of bronchial derivation revealed reduced indicators at the level of large bronchi – in 33.3% of the examined, at the level of medium bronchi – in 63.5% of patients, and at the level of small bronchi – in 88.8% of patients.

Bronchial obstruction (BO) was diagnosed in 51 patients during spirometry. All of them underwent BDT. On the basis of BDT results and clinical data, the presence of concomitant asthma was confirmed in 14 (27.5%) patients with COPD, and these patients were transferred to the ACO group. In 6 (11.8%) patients with COPD, the diagnosis was revised in favor of asthma.

It was important to analyze data depending on FER violations in the middle of groups of patients with different genesis of the disease. In the 1st group of patients with asthma, the percentage of patients with sharp generalized and significant changes in FER was the highest – 14% (8) and 34% (17), respectively. In the III group of patients with COPD, the largest share was patients with moderate changes in FER – 72.4% (21), while in the I group and in the II group of patients with COPD, the number of examined patients with such disorders was almost identical and amounted to 44%, respectively (22) and 48.5% (16). Moreover, the most pronounced obstruction at the level of small bronchi was observed in patients with asthma and ACO, while in patients with COPD, obstruction at the level of medium and small bronchi was equally pronounced.

After the rehabilitation course of treatment, it was recorded that the group with sharp violations of FER decreased to 10%, with significant violations – to 16%, with moderate changes – by 14%. The number of patients with minor lung ventilation disorders increased by 8%. The number of patients with normal FER indicators increased – from 8% to 28% of the total number of patients treated in the 1st group.

In the II group of patients, positive dynamics of indicators of the ventilation capacity of the lungs were also noted. Thus, the number of patients with severe violations of the FER decreased by 6%, with significant – by 9%, with moderate – by only 3%. The number of patients with minor violations of lung ventilation increased by 12.1% (4) and with normal FER indicators – from 9% (3) to 27.3% (9) of the total number of patients treated in the II group.

As for the III group of patients, the dynamics of FER indicators was also pronounced. The number of patients with sharp, significant and moderate changes in lung ventilation decreased by two times. The number of patients with minor violations of FER increased by 17.2% (5), and the number of patients with normal indicators of ventilation increased from 10.3% (3) to 31% (9) of the total number of patients treated in the III group.

The dynamics of FER indicators after the rehabilitation treatment had some differences for each group of patients in relation to the control groups. If in all A groups (control) the increase in FER indicators was insignificant, and in the II-A n/group there was no increase in speed indicators at all, then in all B groups of patients it was significant, but the most pronounced – in I-B group of patients. Comparing

Table 1. Dynamics of FER indicators

	I group				II group				III group			
	I-A gr. N=26		I-B gr. n=24		II-A gr. n=16		II-B gr. N=17		III-A gr. N=15		III-B gr. N=14	
	increase	%to output equal	increase	% to output equal	Increase	% to output Equal	increase	% to output equal	increase	% to output equal	increase	% to output Equal
FVC	4.4	7.4	17.3	26.4	6.7	8.5	11.3	18.2	3.8	5.3	14.4	21
FEV_1	2.7	4.3	16.9	25.5	1.8	2.4	12.7	20.6	6.5	9.4	14.8	22.5
ERV	0.5	4.5	18.5	24.2	0	0	10.4	14.6	4.8	6.2	20.1	28.4
MEF_{25}	0.8	1.4	16.3	25.9	0	0	12.3	22.1	0.4	0.6	15.1	23.8
MEF_{50}	1.0	1.9	17.4	31.3	0	0	10.5	20.9	0.4	0.7	15.0	27.9
MEF_{75}	0.7	1.2.	18.4	36.0	0	0	8.4	18.1	0.1	0.2	17.0	25.3
MEF_{25-75}	0.4	4.2	16.9	29.5	0	0	11.6	22.4	0.2	0.4	14.6	26.3

the increase in FER indicators in the I-B group with the I-A group (control), it can be stated that the increase increased by 3.9 times, FEV1 – by 6.3 times, FVC – by 37 times. The increase in speed indicators was minimal in the I-A group, while it was significant in the I-B group: the increase of MFF25 was 16.3(25.9%), MEF50 – 17.4(31.3%), MEF75 – 18.4(36%), FEF25-75 – 16.9(29.5%) (Table 1).

According to the indicators of lung ventilation in the group of patients with asthma (I-B), it can be stated that the dosed inhalation of salbutamol before the HAT session contributes to better air permeability in all areas of the bronchial tree. Bronchial obstruction in the majority of asthma patients was reversible. A session of GAT in combination with salbutamol proved to be more effective in the rehabilitation treatment of patients with asthma.

Improvement of lung ventilation was also observed in the II-B group, but the indicators were significantly lower than in the I-B group. This is explained by the fact that in patients with COPD, irreversible obstruction of the bronchi with remodeling of the bronchial tree prevailed, and the additional appointment of atrovent did not sufficiently contribute to the improvement of respiratory function and the effectiveness of rehabilitation treatment. Thus, in the II-B group, compared to II-A, the growth of FVC indicators increased by 1.7 times, FEV1 – by 4.2 times, and the ERV – by 4.7 times. The dynamics of speed indicators at the level of large, medium and small bronchi was also significant, the increase ranged from 8.4 to 12.3 (18.1% -22.4%).

The dynamics of FER indicators in the III-B group was also significant, but smaller than in the I-B group and greater than in the II-B group. Thus, the growth of FVC in the III-B group where patients with ACO were treated compared to III-A increased by 2.1 times, FEV1 – by 4.9 times, and ERV – by 9.1 times. The increase in velocity indicators at the level of large and medium bronchi was at the level of 15.1 and 15.0 (23.8% and 27.9%, respectively). The increase in air permeability at the level of small bronchi – MEF75 was significant and amounted to 17.0 (25.3%), which is 2 times more than in II-B n/group in patients with COPD, but less than in I-B group in patients with asthma. Which proves the thesis about the special nature of the obstructive syndrome in patients with ACO.

DISCUSSION

COPD and asthma are two diseases that can coexist in the same patient and under the influence of different etiological factors can acquire features of each other [28, 29]. Thus, asthma under the influence of tobacco smoking can acquire signs of COPD with fixed airflow obstruction, with subsequent development of pulmonary emphysema. Smoking is a prerequisite for the development of ACO [30].

Asthma and COPD are two diseases of the respiratory system, the main clinical and functional manifestation of which is bronchi-obstructive syndrome. These diseases arise as a result of various etiological factors and are characterized by various pathogenetic mechanisms. Thus, the development of asthma is often based on eosinophilic inflammation of the respiratory tract. As a response to the action of pathological factors, hyperreactivity of the bronchi is formed. A feature of bronchial obstruction is its reversibility. The basis of the development of COPD is mostly neutrophilic inflammation of the respiratory tract in response to tobacco smoke, negative environmental and household factors with damage to the bronchi and a frequent complication – emphysema. Clinical and functional manifestations include irreversible or partially reversible bronchial obstruction [2, 4, 7].

Scientific studies of recent years have shown that in a certain category of patients, a combined pathology of asthma and COPD may develop. ACO is a heterogeneous condition that describes patients with persistent airflow limitation and clinical and inflammatory features of both asthma and COPD [16].

We considered ACO as a disease with heterogeneity of clinical, diagnostic and laboratory features that can

be used in the diagnosis of ACO. According to our data, patients with ACO were clinically similar to patients with asthma only and COPD only, but symptoms, quality of life, and non-respiratory comorbidities were more similar to COPD. It was also noticed that patients with ACO have a more severe course of exacerbations than with asthma and COPD separately [18, 28].

There are still many disputes among scientists regarding the existence of ACO as a separate nosology. In 2020, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [31] stopped referring to ACO due to the lack of an existing commonly accepted definition [32]. However, despite this, the diagnosis of this condition remains extremely important, as these patients experience deterioration more quickly than with asthma or COPD alone. Treatment in general and rehabilitation, in particular, of patients with ACO has not yet been determined, because patients with this diagnosis are traditionally excluded from clinical research [16, 18, 20, 33]. Today, many criteria are proposed for the diagnosis of ACO, but there is no generally accepted standard [19].

According to our data, ACO is formed jointly. Some patients are diagnosed with asthma with a classic «allergic march» in childhood, and later in adulthood, the picture of the disease changes due to smoking and other pathogenic factors. Neutrophil inflammation begins to be noted, the ventilatory function of the lungs decreases. Bronchial obstruction acquires signs of irreversibility. The response to inhaled glucocorticoid therapy worsens, which is confirmed by literature data [28, 30, 33]. Other patients with COPD may develop hay Fever first, and then asthma. All signs of asthma are present: eosinophilia, an increase in IgE in the blood, and bronchial obstruction acquires signs of reversibility, which is consistent with other studies [34, 35].

Patients with ACO with an average age of 43 years participated in our study. These data are fully consistent with the literature data [6, 34]. When analyzing the anamnestic data among patients with ACO, asthma was the primary cause in the development of the pathological process in one in five, and COPD in four out of five, which is confirmed by other authors [16, 18, 19, 20]. In 50% of patients with COPD, the diagnosis of ACO was confirmed by additional diagnostic tests and clinical criteria [28, 35].

Since ACO can combine an inflammatory process in bronchial tubes such as asthma and COPD, the risk of non-reversible obstruction increases significantly [36, 37]. To determine the reversibility of bronchial obstruction, our study used spirometry data before and after bronchodilators – bronchodilation test (BDT), forced expiratory volume in 1 second FEV1, vital lung capacity FVC and the ratio of these parameters FEV1/FVC [38, 39]. Performing BDT is an integral component of diagnostic measures and significantly improves the quality of diagnosis of COPD and asthma. The diagnostic quality of this test in general practice is low. Carrying out adequate spirometry with BDT made it possible to rule out COPD in 50% of patients with a diagnosis established by the primary care unit. Among them, asthma occurred at 19.7%. In 30.5% of patients, the diagnosis of COPD required clarification. These patients were included in the III group of patients with ACO, which is usually not included in clinical studies due to the heterogeneity of symptoms [28, 30, 31].

After the spirometry study was conducted, it was established that in the group of asthma patients, reversible bronchial obstruction was characteristic to a greater extent, in the group of patients with COPD – irreversible bronchial obstruction, in the group of patients with ACO – not completely reversible obstruction [30, 40]. Due to the different types of obstruction in each group of patients, different bronchodilators were proposed in the form of a metered dose aerosol inhaler (MAI) [41]. Salbutamol was offered to the group of patients with asthma (II-B) before the NAT session. Salbutamol – β2-adrenomimetic, which is effective in the treatment of reverse obstruction, especially in patients with asthma [21]. Patients with COPD (II-B) took DAI atrovent (ipratropium bromide). Atrovent is an M-cholinergic that is effective in the treatment of patients with COPD [22]. Patients in the III-B group (patients with ACO) were offered a combined drug – combivent (salbutamol + ipratropium bromide), which affects M-cholinergic receptors of the tracheobronchial tracts, mainly in large and medium-sized bronchi, as well as β2-adrenoceptors of the smooth muscles of the bronchi and bronchioles [23].

After a course of rehabilitation treatment, lung ventilation improved in all groups of patients compared to controls. The dynamics of FER indicators after the rehabilitation treatment had some differences for each group of patients in relation to the control groups. If in all A groups (control) the increase in FER indicators was insignificant, and in the II-A group the increase in speed indicators was not ascertained at all, then in all B groups of patients it was significant, but differed in %. It was most pronounced in the I-V(A) group of patients. The percentage increase of FER (air permeability) indicators to the initial level ranged from 24.2% to 36.0%. The largest increase was noted at the level of small bronchi and amounted to 36.0%. In the III-B (ACO) group, the percentage increase was somewhat lower and ranged from 21% to 28.4%, but was equal for all indicators. The smallest increase in FER indicators was observed in the II-B B (COPD) group.

Thus, medical care for patients with ACO requires a holistic approach, which is not found in guidelines worldwide. This approach should include both pharmacological and non-pharmacological methods of influencing the pathological process. Non-pharmacological methods of influence include physical activity, pulmonary rehabilitation, smoking cessation and diet [37].

CONCLUSIONS

Thus, a differentiated approach to the use of fixed-dose aerosol inhalers increases the effectiveness of rehabilitation treatment under conditions of halo aerosol therapy in patients with various genesis of bronchi-obstructive syndrome.

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CONFLICT OF INTEREST
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LUNG PROTECTIVE EFFECTS OF CLOPIDOGREL IN POLYMICROBIAL SEPSIS

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Abstract

Aim: The goal of this experiment was to examine if Clopidogrel might protect the lungs during sepsis by modulating the inflammatory and oxidative stress markers.

Materials and Methods: Twenty-four adult male Swiss-albino mice aged 8-12 weeks, with a weighing of 20-30 g, were randomized into 4 equal groups (n=6): sham (Laparotomy without cecal ligation and puncture [CLP]), CLP (laparotomy plus CLP), vehicle (DMSO 1 hour prior to CLP), Clopidogrel (50 mg/g IP 1 hour before to CLP). ELISA was used to assess Lung tissue levels of pro-inflammatory and oxidative stress markers.

Results: F2 isoprostane levels were significantly higher in the sepsis group ($p<0.05$) in comparison with sham group, while Clopidogrel was considerably lower ($p<0.05$) in the inflammatory and oxidative stress markers in comparison to sepsis group. Histologically, all mice in the sepsis group had considerable ($p=0.05$) lung tissue damage, but Clopidogrel considerably decreased lung tissue injury ($p=0.05$).

Conclusion: Clopidogrel was found to reduce lung tissue cytokine concentrations (IL-1, TNF a, IL-6, F2 isoprostane, GPR 17, MIF) in male mice during CLP-induced polymicrobial sepsis by modulation of pro-inflammatory and oxidative stress cascade signaling pathways, to the best of our abilities, no study has looked at the effect of Clopidogrel on MIF levels.

KEY WORDS: lung protective effects, effects of Clopidogrel, polymicrobial sepsis

INTRODUCTION

Despite improvement in supportive care, sepsis continues to be a major public health concern; even survivors impose significant medical and financial burdens on health-care systems. Every year, more than one in every four individuals worldwide dies from sepsis [1, 2]. Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [3, 4]. Sepsis can occur after any infection, including bacterial, parasitic or viral diseases such as malaria or COVID-19 [5]. The identification of key products as cytokines and incorporating these molecules into laboratory animals could result in a clinical symptoms resembling sepsis, fueled the search for specific agents that can inhibit, or reduce the possible effects of [6] regulation of the innate immune response by monocyte to pathogens through generating inflammatory cytokines such as interleukin-6 (IL-6) and Interleukin-1β [7]. In some research for example, interleukin-6 has been linked to tissue inflammation-induced tissue damage, and Interleukin-1β is associated with pulmonary inflammatory disease and emphysema [8, 9]. Clopidogrel is an irreversible P2Y12 receptor antagonist that is taken orally and suppresses platelet aggregation. Clopidogrel is an important treatment choice for patients with ACS, recent MI, or stroke.

AIM

The goal of this experiment was to examine if Clopidogrel might protect the lungs during sepsis by modulating the inflammatory and oxidative stress markers.

MATERIALS AND METHODS

The study was carried out at the Faculty of Medicine/ University of Kufa's Department of Pharmacology and Therapeutics, as well as the Middle Euphrates Unit for Cancer Research. 24 mature Swiss albino male mice with weight 21-29 gram and age 8-12 weeks have been collected derived from Animal Shelter in the Science's College in Kufa's University. The mice's` maintained in the animal shelter of University of Kufa at 25°C temperature as well as humidity of 60-65 %, with a cycle of day light 12 hour: dark 12 hour. The animals have been divided to four groups, each group with six male mice as shown below:

Sham: the mice in the group have been anesthetized and operated laparotomy surgery but did not have CLP;

Sepsis (CLP) the mice in the group had their cecums ligated then punctured;

Vehicle: All animals received dimethyl sulfoxide (DMSO) in equal volumes and intraperitoneal injection 1 hour before CLP treatment;

Clopidogrel pre-treated group: one h prior to CLP, the mice in the group were given a 50 µg/g Clopidogrel IP inject.

EXPERIMENTAL PROCEDURE

In brief, mice have been anesthetized intraperitoneally with 100 mg/kg ketamine and 10 mg xylazine. Incision with a 1.5 cm in the midline was used for abdominal laparotomy, and the cecum was exposed. Then, the cecum have been ligated right below the ileocecal valve then doubly punctured

Table 1. Scale of lung tissue damage.

Score		Damage
0	Normal	normal architecture
1	Mild	25 percent damage
2	Moderate	25-50 percent damage
3	Severe	50-75 percent damage
4	Highly severe	75-100 percent damage

with Gram-22 needle prior to the returning to its location. The abdomen have been then sutured with a 5/0 surgical suture size [10]. Mice were checked every 4 hours for 24 hours prior to returning with unlimited access to their cages access to drink and food [11].

PREPARATION OF DRUGS

Clopidogrel was bought from Med. Chem. Express in the United States and formulated in 100 mg/ml of (DMSO and SBE). It was administered at a dose of 50 mg/g i.p [12] The mice's lung was excised 24 hours after CLP. Tissue samples were divided into two portions, with half of the samples frozen at -80C until ELISA analysis. The remaining samples were preserved in 10% formalin for histological examination.

TISSUE SAMPLE PREPARATION FOR HISTOPATHOLOGY

To eliminate red blood cells or clots, lung tissues obtained following mouse sacrifice were rinsed with cold isotonic sodium chloride solution 0.9%. The tissue was then fixed in 10% formalin and processed in paraffin blocks. Following fixation, the specimens were dehydrated by immersing them in ethanol for two hours for each (70, 80, 90, and 100%) to remove any remaining formalin or H2O from the samples. They have been then cleaned with xylene in order to take away the alcohol before seeingping with paraffin wax (an embedding agent). Histological sections from all groups were evaluated to semi-quantify the difference in lung damage. The histopathology examination was carried out at magnifications ranging from X100 to X400 [13] and assessed through the following percentage of tissue damage (Table 1).

STATISTICAL ANALYSIS

For statistical analysis, SPSS with version 26 was used. The data has been presented as Mean ± SEM. For multiple group comparisons, ANOVA have been used, followed by a post-hoc test with Bonferroni correction. The Kruskal-Wallis test have been performed to examine if the statistical difference between the several groups significant as mean score for histological alterations in lung tissue, P value 0.05 was considered statistically significant.

ETHIC STATEMENT

The present work followed the guidelines of the Association for Laboratory Animal Science's Guide for the Care and Use of Laboratory Animals. The Animal Care Committee of Al-Kufa University acknowledged all customs and concerns for living things. All mice were made while under the ketamine and xylazine-induced anesthesia, and every effort was made to lessen each person's suffering, Kufa University, 148/2022.

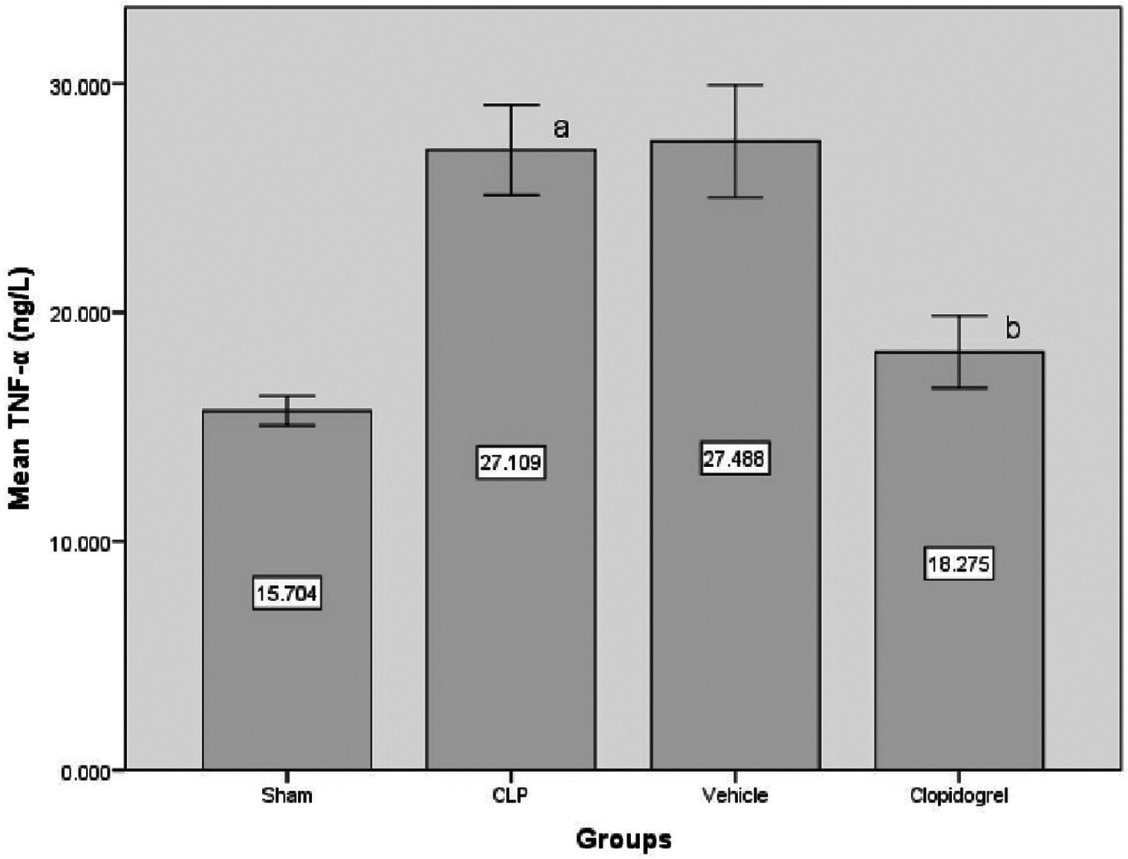

Fig. 1. TNFα (ng/L) ± SEM mean lung tissue level in the groups; sham *vs.* sepsis (p-value=0.00001), Clopidogrel *vs.* sepsis and vehicle (p-value=0.0001)

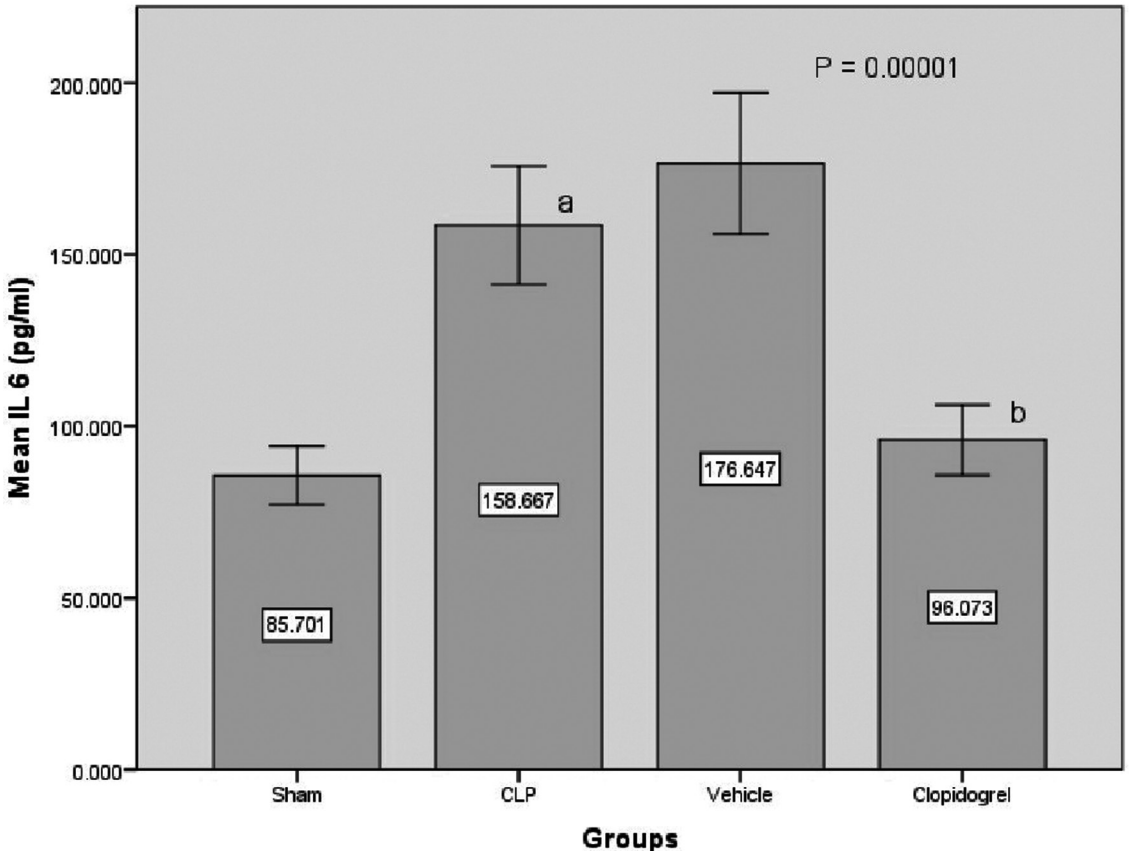

Fig. 2. IL6 (pg/ml) ± SEM mean lung tissue level in the groups; in comparing sham *vs.* sepsis (p-value = 0.00001), Clopidogrel *vs.* sepsis and vehicle (p-value = 0.0001).

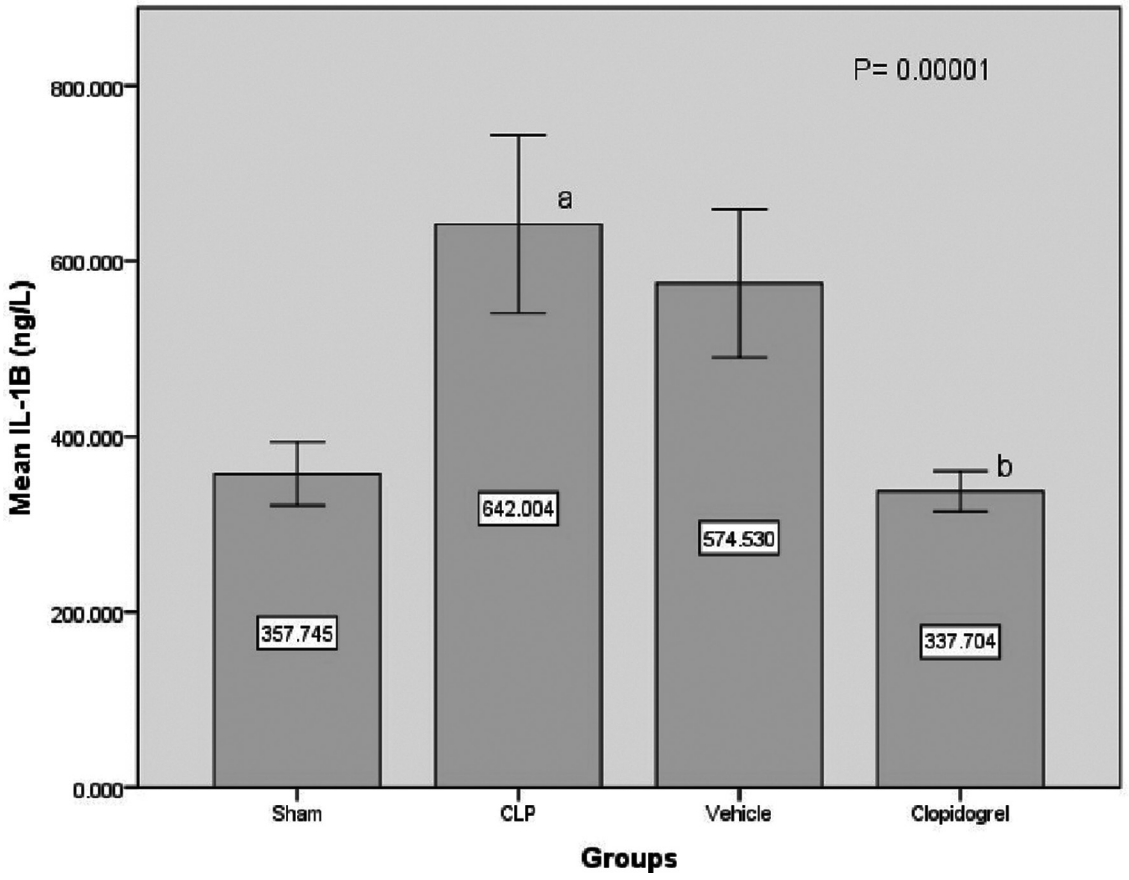

Fig. 3. IL1β (ng/L) ± SEM Mean lung tissue level in the groups in the completion of the study; in comparing sham *vs.* sepsis (p-value=0.00001), clopidogrel *vs.* sepsis and vehicle (p-value=0.0001)

RESULTS

CLOPIDOGREL DECREASED INFLAMMATORY IL-6, IL-1B, AND TNFA MARKERS IN LUNG TISSUE

In the comparison the sepsis group to the sham group, TNFα level have been significantly increased in the sepsis group ($p<0.05$). While in comparing sepsis group, TNFα lung level in Clopidogrel treated group was lower (Fig.1). IL-6 and IL-1β lung tissue level were considerably rose in the sepsis group in comparison with the sham one ($p<0.05$). While in comparing the sepsis group, clopidogrel pre-treated group revealed significantly lower levels of IL-6 and IL-1β proinflammatory marker (Fig.2, Fig.3).

CLOPIDOGREL DECREASED GPR17 MARKER IN LUNG TISSUE

The GPR 17 levels in the tissue of the lung were considerably increased in the control group in comparison with the sham group ($p<0.05$). When comparing with the sepsis group, the clopidogrel pre-treated group revealed significantly reduced GPR 17 expression (Fig.4).

CLOPIDOGREL DECREASES OXIDATIVE STRESS (F2-ISOPROSTANE) IN LUNG TISSUE

In opposed to sham group, in sepsis group the mice had a significant increase in F2-isoprostane level. In Comparing with the sepsis group, clopidogrel pre-treatment decreased the amount of F2-isoprostane lung level (Fig.5).

CLOPIDOGREL DECREASES MIF IN LUNG TISSUE

In comparison to sham group, sepsis group mice had a significant increase in MIF level in lung tissue. In contrast to the control group, Clopidogrel pre-treatment decreased the MIF levels in lung tissue (Fig.6).

CLOPIDOGREL DECREASES CASPASES 11 IN LUNG TISSUE

In comparison with the sham group, the sepsis group mice had a considerable caspase11 level increase in the tissue of the lung in Comparison with the sepsis group, Clopidogrel pre-treated group reduce caspases 11 lung tissue level (Fig. 7).

CLOPIDOGREL MINIMIZED LUNG INJURY

Normal lung tissue architecture was showed in the sham group histopathological examination, in sepsis and vehicle groups, development of congestion, perivascular inflammation, interstitial oedema, and localized extravasations of RBCs. Clopidogrel pre-treated group, the showed moderate architectural alterations in comparison with the sham group (Fig. 8).

DISCUSSION

Sepsis is an organ failure illness caused by an immune reaction to the infection, as a result, an uncontrollable inflammatory response and immune suppression. The

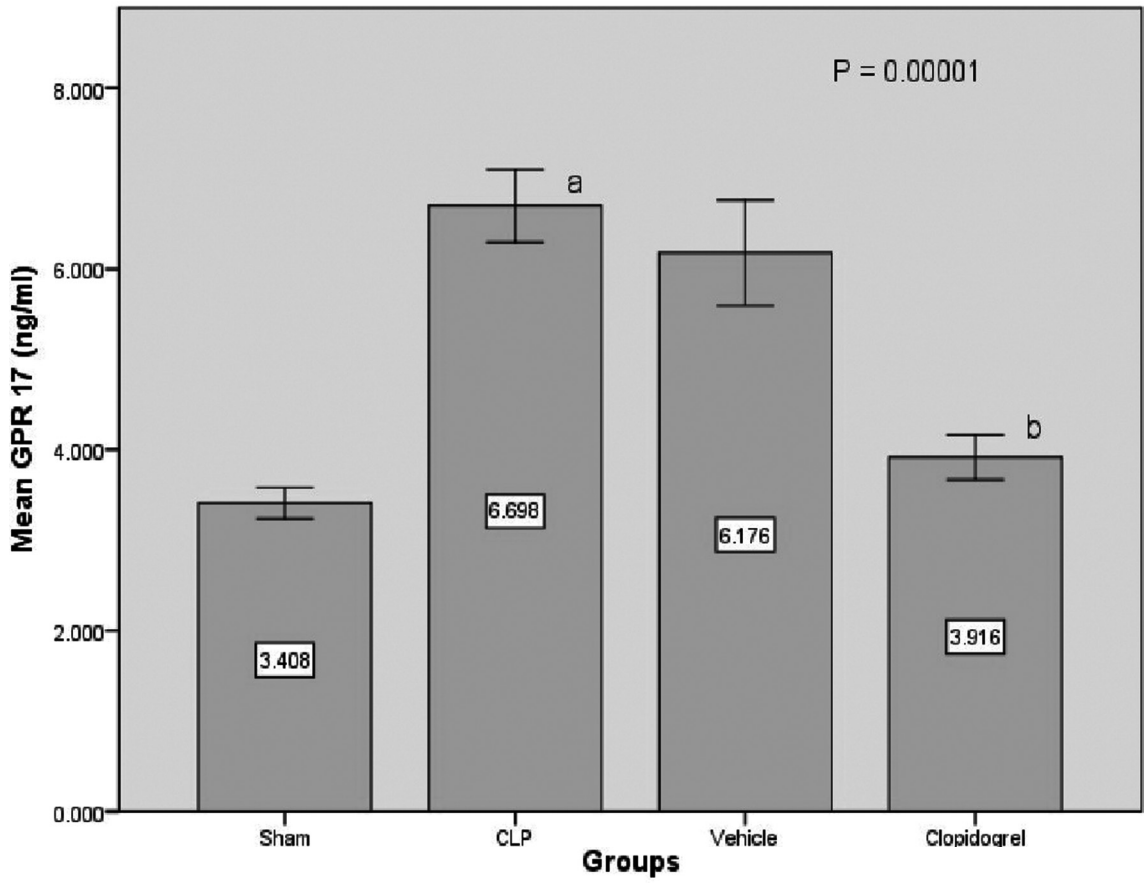

Fig. 4. GPR17 (ng/ml) ± SEM mean lung tissue level in the groups; sham *vs.* sepsis (p-value=0.00001), clopidogrel *vs.* sepsis and vehicle (p-value=0.0001)

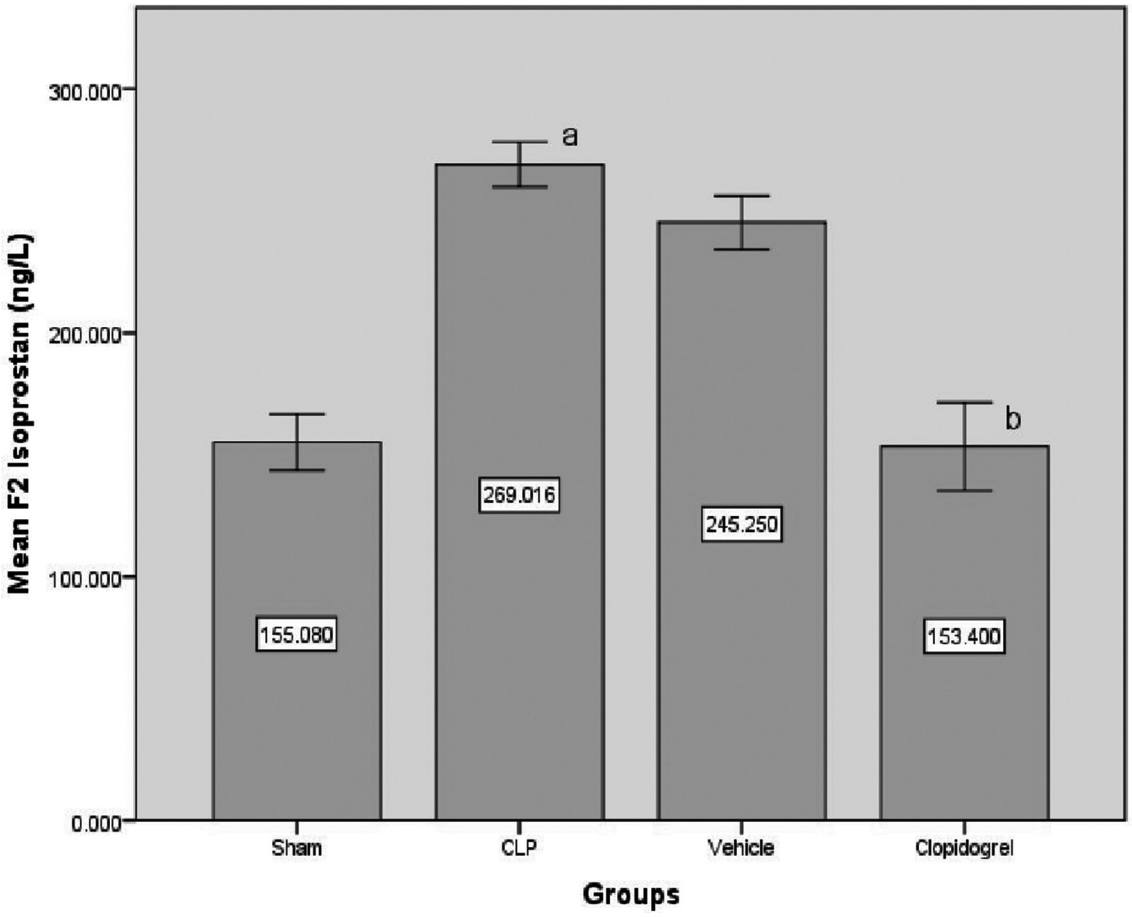

Fig. 5. F2 isoprostane (ng/L) ± SEM mean lung level in the groups; sham *vs.* sepsis (p-value=0.00001), clopidogrel *vs.* sepsis and vehicle (p-value=0.0001)

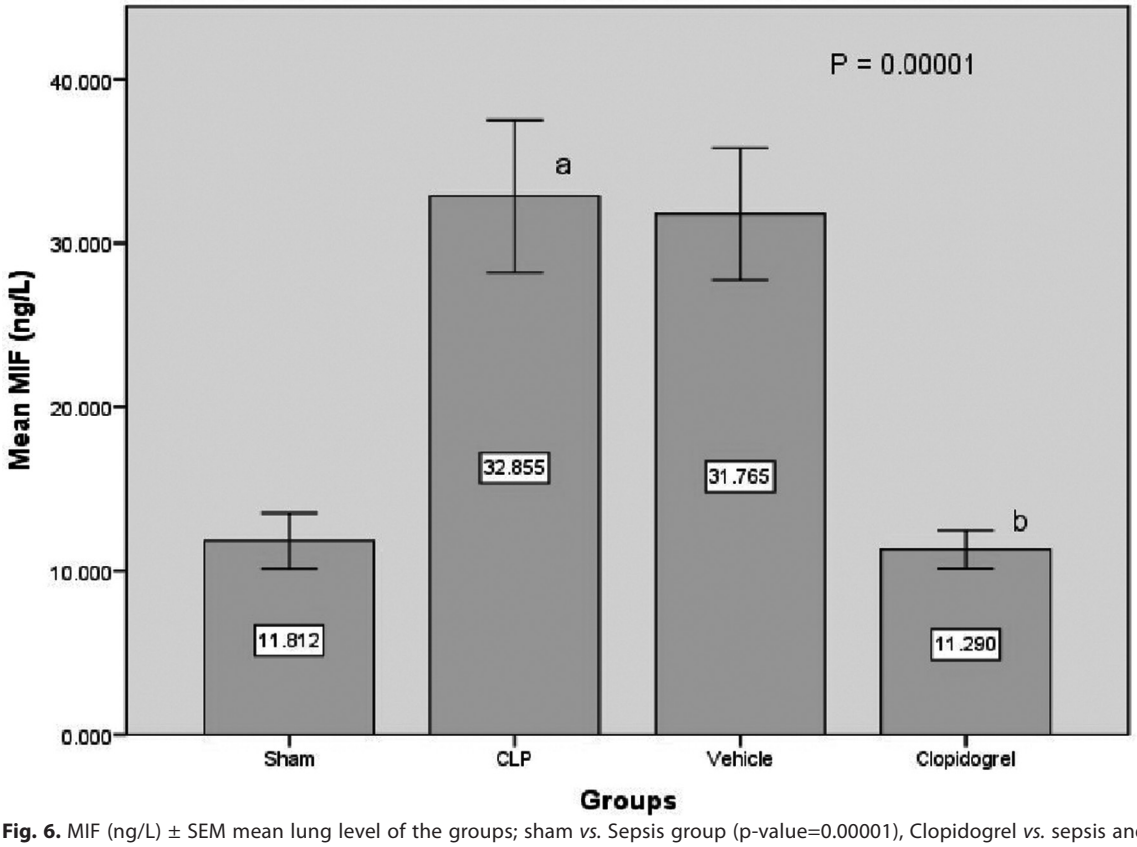

Fig. 6. MIF (ng/L) ± SEM mean lung level of the groups; sham *vs.* Sepsis group (p-value=0.00001), Clopidogrel *vs.* sepsis and vehicle group (p-value=0.0001)

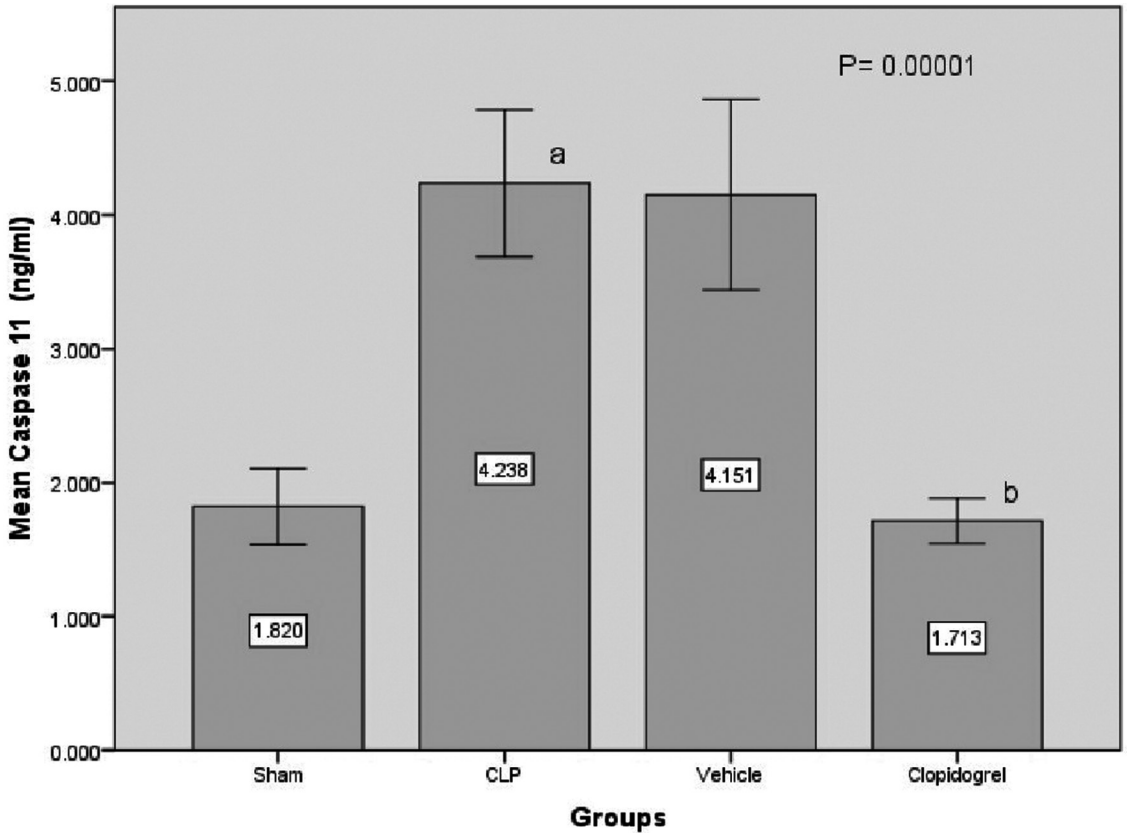

Fig. 7. Caspases 11 (ng/L) ± SEM mean lung tissue level; sham *vs.* sepsis group (p-value=0.00001), Clopidogrel *vs.* sepsis and vehicle group (p-value=0.0001)

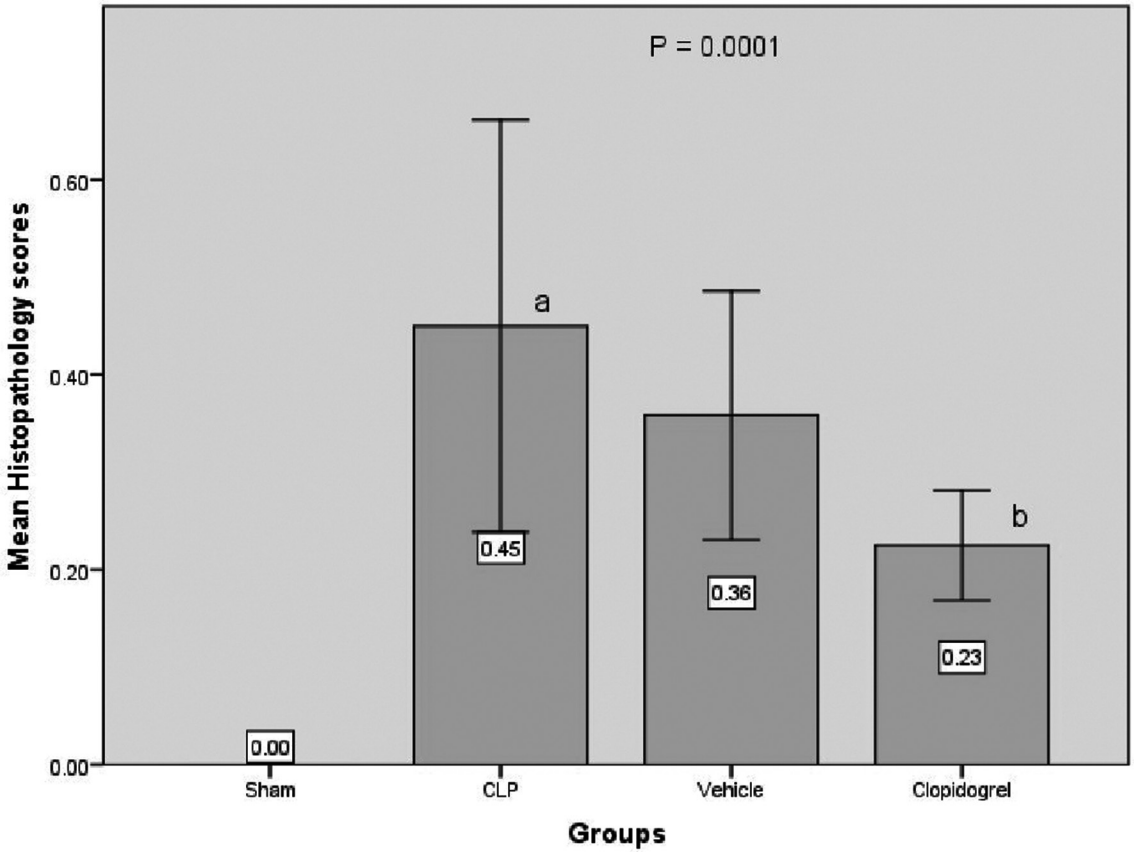

Fig. 8. Histopathological results of the four test groups were as follows: sham *vs.* sepsis and vehicle (p-value=0.0001), Clopidogrel *vs.* sepsis (p-value=0.0001), Clopidogrel *vs.* sepsis (p-value=0.0001)

severity of the inflammatory response as well as its prolonged leukocyte activation can influence ARDS clinical course, since pro-inflammatory activated cytokine may enter the circulation as well as causing multiple organ dysfunction in ARDS due to alveolar compartmentalization loss [14]. The cytokines, particularly IL-1β, IL-6, TNFα, as well as caspase11, play critical roles in lung dysfunction [15].

SEPSIS EFFECT ON (IL1B, IL-6 AND TNFA)

The present study has shown that considerably higher level of TNFα, IL1β and IL-6 in lung tissue in the sepsis and vehicles groups in comparison with the sham. In accordance with other findings [15, 16] state that endotoxic shock causes a considerable rise in IL-1β and IL-6 levels. Increased pro-inflammatory cytokine levels can also affect microcirculation [17], throughout this period of severe systemic inflammation, the principal harm induced by sepsis in the course of ARDS is hyper permeability of pulmonary microvasculature, and consequently a plasma exudate fills the alveoli [18]. Furthermore, alveolar oedema is caused by increased exudate in alveolar gaps, in addition to the loss of alveolar epithelial cells caused by apoptosis and necrosis caused by sepsis. The hallmark of the acute phases of ARDS causing damage spread.

EFFECT OF CLOPIDOGREL ON PROINFLAMMATORY CYTOKINES (IL1B, IL-6, TNFA)

The present study found that Clopidogrel-pretreated groups had significantly lower lung tissue levels of proinflammatory cytokines (IL1β, IL-6, and TNFα) in comparison with the sepsis group. Following CLP [19], Clopidogrel pretreated group resulted in significant decrease in proinflammatory TNFα and IL1B cytokine levels. Some studies have shown platelet-leukocyte interactions play an important role during sepsis and have been implicated in acute lung injury [2]. Hadi et al. [19] showed that platelet leukocyte complexes were decreased after clopidogrel pretreatment compared to sepsis group. As a result, suppression of platelet-leukocyte interactions is assumed to be one of the primary ways through which clopidogrel affects inflammation.

SEPSIS EFFECT ON F2 ISOPROSTANE

According to the study, F2 isoprostane level in the CLP group have been significantly greater than the sham group. Increased reactive oxygen species and oxidative stress generation are play important role in the inflammatory response initiation and maintenance. The findings of this study are consistent with those of Hadj Abdallah et al. [20] who demonstrated that oxidative stress has a significant influence in sepsis in both mitochondrial and endothelial dysfunction, and that oxidative stress biomarkers would be useful in an optimal sepsis diagnosis and prognosis.

CLOPIDOGREL EFFECT F2 ISOPROSTANE

The present study found that clopidogrel-treated group showed a greater decrease in the tissue lung level of oxidative stress F2 isoprostane as compared with sepsis and vehicle group. Hadi et al. [21] found that clopidogrel decrease biomarkers of oxidant stress in patient with coronary artery disease. The research also line with the previous study in which clopidogrel decrease vascular oxidative stress induced by angiotensin 2 during the stages of CVDs, there is a tight link between unregulated platelet activity and oxidative stress [22, 23] however; the exact mechanism of p2y12 receptor and oxidative stress is not fully investigated.

EFFECT OF SEPSIS ON THE LEVEL OF CASPASES 11

The present study has shown that significantly higher lung tissue level of the pyroptosis-related proteins caspases-11 in sepsis and vehicles groups in comparison with the sham group [24] and obtained similar result [25] also line with the previous study in which caspase-11-dependent pyroptosis in the lungs of the CLP-induced ALI model increased significantly when compared to the sham group [26] showed higher expression of caspase-11after LPS injection. Previous research has shown that caspase-11 functions as an endogenous receptor for cytosolic LPS recognition in order to induce pyroptosis [27]. Caspase-11 knockout mice, but not caspase-1 knockout mice, are resistant to LPS-induced septic shock [28-29], however, the metabolic regulation of the caspase-11 inflammasome remains unknown [30].

EFFECT OF CLOPIDOGREL ON THE LEVEL OF CASPASES 11

The study showed that Clopidogrel-treated group showed a greater decrease in the tissue lung level of caspases-11 as compared with sepsis and vehicle group. There is no previous look about the effect of Clopidogrel on the level of caspases-11

EFFECT OF SEPSIS ON THE LEVEL OF GPR 17

The present study has shown that considerably higher lung level of GPR17 in the sepsis and vehicles groups in comparison with the sham. Al-Amran et al. [31] showed similar result in which there is up regulation of the level of GPR17 receptor in a significant manner. There is growing evidence that modulating GPR17 can improve inflammatory responses and injury [15]. Inhibiting GPR17, for example, suppressed microglial activation and alleviated inflammation caused by ischemic stroke in rats [32], Inhibiting GPR17 also protects against myocardial fibrosis caused by cardiac ischemia [33].

EFFECT OF CLOPIDOGREL ON THE LEVEL OF GPR 17

The current study found that clopidogrel-treated group showed a greater decrease in the tissue lung level GPR17 as compared with sepsis and vehicle group. Previous research found that administering a P2Y12 antagonist, specifically cangrelor, reversed the increase in GPR17 caused by CLP while also alleviating the inflammatory response [33]. However, the relationship between P2Y12 receptor and GPR17 is still unknown. To the best of our knowledge, there is no data available about the effect of clopidogrel on GPR17 in lung injury sepsis.

EFFECT OF SEPSIS ON THE LEVEL OF MIF

The present study has shown that considerably higher lung tissue level of MIF in sepsis and vehicles groups in comparison with the sham group. Our finding were

consistent with finding of previous study [34]. MIF is a pro-inflammatory cytokine that is abundant in macrophages, vascular ECs, tissue epithelial cells, and tumor cells [35]. MIF has an effect on TLR4-mediated innate immunity, which cause recruitment of immune cells to inflammatory area, activation of inflammatory pathways, and induction of immune cell differentiation.

EFFECT OF CLOPIDOGREL ON THE LEVEL OF MIF

The current study found that Clopidogrel-treated group showed a greater decrease in the tissue lung level of MIF as compared with sepsis and vehicle group. MIF, a chemokine-like cytokine, is secreted from activated platelets, indicating that platelets are a previously unknown source of MIF in inflammatory processes. Because MIF contributes significantly to the chemotactic capacity of stimulated platelet supernatants, this suggests a role for platelet-derived MIF in atherogenic cell recruitment [36]. To the best of our abilities, no study has looked at the effect of clopidogrel on MIF levels.

CONCLUSIONS

Clopidogrel was found to reduce lung tissue cytokine concentrations (IL-1, TNF a, IL-6, F2 isoprostane, GPR 17, MIF) in male mice during CLP-induced polymicrobial sepsis by modulation of pro-inflammatory and oxidative stress cascade signaling pathways, to the best of our abilities, no study has looked at the effect of Clopidogrel on MIF levels.

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IS FECAL CALPROTECTIN DETERMINATION USEFUL FOR PATIENTS WITH METABOLIC ASSOCIATED FATTY LIVER DISEASE?

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ABSTRACT

Aim: To investigate the possible relationship between fecal calprotectin (FC) level and ultrasound indicators of steatosis and fibrosis wich defined by attenuation coefficient (AC) and liver stiffness (LS) from two-dimensional (2D) shear-wave elastography (SWE) in patients with metabolically associated fatty liver disease (MAFLD).

Materials and Methods: The study included 110 persons with MAFLD; mean age 51.3±4.8 years, 65 (59.1%) men. There were used laboratory, sonography and statistical methods.

Results: Stage S1 of steatosis was diagnosed in 42 (38.2%), S2 - in 56 (50.9%), S3 - only in 12 (10.9%) MAFLD patients. The carbohydrate metabolism disorders were found in 62 (56.4%); 38 (34.5%) patients among them suffered from type 2 diabetes. The lipid metabolism disorders were diagnosed in the vast majority of patients included in this study. The minimal excess of fecal calprotectin (FC) was detected in 72 MAFLD patients (65.5%), the moderate increase of FC was found in 12 persons, the FC more than 10-fold excess of the norm was observed in only 8 MAFLD patients. FC levels were significantly elevated in MAFLD patients with a S2-S3 compared to those with a S1 (75.8 [42.9–112.1] *vs.* 46.3 [28.2–65.4], $p<0.01$).

Conclusions: Fecal calprotectin levels are significantly elevated in patients with MAFLD. Future studies are warranted to establish the definitive role and clinical utility of FC as a potential biomarker of probably liver steatosis as well as other diseases associated with methabolic syndrome and its complications.

KEY WORDS: fecal calprotectin, inflammation biomarker, steatosis, two-dimensional shear-wave elastography, ultrasound steatometry

INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is quite common in the modern population. It is currently the most common liver disease worldwide, affecting over one-third of the population [1]. This disease is most often caused by insulin resistance, is one of the components of the metabolic syndrome and is combined with various disorders of substances in the body. That is why it was called MAFLD instead of NAFLD. The course of MAFLD is most often asymptomatic or mildly symptomatic, and the complaints of the vast majority of such patients are nonspecific and vague, such as heaviness (rarely muffled non-intense pain) in the right hypochondrium or discomfort in the abdomen as a result of bloating and excessive gas formation.

Flatulence is the symptom that makes the vast majority of such patients consult a doctor. Subsequently, during the examination of most of such persons, it turns out, as an accident, that the person's diagnosis is MAFLD. For patients with abdominal distension, it is important to investigate the cause of this condition, as flatulence can occur due to a variety of reasons. Among them are: pancreatic enzyme insufficiency, biliary dysfunction, inflammatory bowel diseases, helminthiasis and others. For such a cohort of patients, multiparametric ultrasound examination of the liver is important, but currently there is a lack of data from multicenter studies on the role of this instrumental examination in the diagnosis of MAFLD.

It is known about the existence of the "intestine-liver" pathogenetic axis [1]. Accumulating evidence points to a significant role of inflammation as a one of pathophysiological driver of MAFLD progression [2]. In the pathogenesis of MAFLD, hepatocytes suffer from cellular stress. Neutrophils granulocytes play a significant role in the pro-inflammatory cascades [3]. Calprotectin is a calcium- and zinc-binding protein dimer (also known as the S100A8/S100A9 complex) that is present in the cytosol of neutrophilic granulocytes [4]. Upon inflammation, calprotectin is actively secreted by neutrophils as part of their stress response [5, 6]. Fecal calprotectin (FC) is clinically used as a biomarker of active intestinal inflammation in patients with inflammatory bowel disease (IBD) [7]. Given the role of hepatic infiltration of neutrophils and systemic inflammation in MAFLD, calprotectin levels may also reflect the inflammation in the pathogenesis of MAFLD [1]. However, only a limited number of clinical studies have been performed examining calprotectin as biomarker in subjects with MAFLD or NAFLD, and conflicting results have been reported [8-10]. Neutrophilic inflammation

plays a known role in the pathogenesis of MAFLD [11].

But does fecal calprotectin change in patients with MAFLD? Does FC correlate with indicators of steatosis and fibrosis in case of presence of MAFLD?

AIM

Therefore, the purpose of our work was to investigate the possible relationship between fecal calprotectin level and ultrasound indicators of steatosis and fibrosis which defined using the attenuation coefficient (AC) from attenuation imaging (ATI) and liver stiffness (LS) and dispersion slope (DS) from two-dimensional (2D) shear-wave elastography (SWE) in patients with metabolically associated fatty liver disease.

MATERIALS AND METHODS

The study included 110 subjects with MAFLD who underwent multiparametric ultrasound examination of the liver with steatometry (measurement of attenuation coefficient) and two-dimensional shear wave elastography (2D-SWE) (measurement of liver stiffness). Each patient, in addition to ultrasound, underwent a general clinical examination, which included some biochemical blood parameters (ALT, AST, ALP, GGT, total and direct bilirubin, total protein, cholesterol, triglycerides, glucose, glycosylated hemoglobin, thyroid-stimulating hormone), calculation of the body mass index (BMI), atherogenicity coefficient (AC) and the insulin resistance coefficient (IR-HOMA)

The mathematical apparatus of the study included the following formulas:

1. BMI = m / h^2, where BMI – body mass index, m – weight (kg), h – height (m)
2. AC = (total cholesterol - HDL) / HDL, where AC – atherogenicity coefficient, HDL - high-density lipoproteins
3. IR-HOMA = fasting blood glucose * fasting blood insulin) / 22.5

Multiparametric ultrasound investigation of the liver was carried out using the SONEUS P7 (Ultrasign, Kharkov, Ukraine) and a C1-5 convex sensor with frequencies of 1-5 MHz. We prospectively investigated patients with MAFLD by two-dimensional shear-wave elastography (2D-SWE) with associated controlled attenuation parameter (CAP) as part of a routine clinical investigations. MAFLD was defined as CAP ≥ 2.20 dB/cm. Significant liver fibrosis (stage 2 or higher out of 4) was defined as LS measurement ≥7.0 kPa.

The presence of fibrosis in the liver tissue was assessed using two-dimensional shear wave elastography, measuring the Young's modulus of the liver (liver stiffness, LS) in kilopascals (kPa) and the speed of the shear wave during the passage of ultrasound through the liver tissue (attenuation coefficient) in m/s. Staging of fibrosis was carried out according to the following criteria:

- F0 - absence of fibrosis: LS 6.5 kPa and less; shear wave speed 1.47 m/s and less
- F1-F2 – fibrosis without septa formation: LS 6.6-6,9 kPa; shear wave speed 1.48-1.52 m/s
- F2 – fibrosis with single septa: LS 6,9-7,5 kPa; shear wave speed 1.52-1.58 m/s
- F2-F3 - LS 7,5 -8,2 kPa; shear wave speed 1,58-1.65 m/s
- F3 – fibrosis with multiple septa, without cirrhosis: LS 8.2-9.3 kPa; shear wave speed 1.65 - 1.76 m/s
- F4 – fibrosis with multiple septa, with cirrhosis: LS is more than 9.3 kPa; the speed of the shear wave is more than 1.76 m/s

To assess the degree of steatosis, we performed ultrasound liver steatometry and measured controlled attenuation parameter (CAP). Staging of steatosis was carried out according to the following criteria:

- stage S0 (no steatosis) – when CAP less than 2.2 dB/cm
- stage S1 (mild steatosis) – when the fatty infiltration in 5 – 33% of liver cells and CAP from 2.20 to 2.25 dB/cm
- stage S2 (moderate steatosis) – when the fatty infiltration in 33-66% of liver cells and CAP from 2.3 to 2.90 dB/cm
- stage S3 (severe steatosis), when more than 66% of liver cells are infiltrated by fat occupies, CAP in this stage is more than 2.9 dB/cm

Fecal calprotectin (FC) was determined in the laboratory by the solid-phase enzyme-linked immunosorbent assay method. A calprotectin level in feces less than 50 μg/g was considered as normal.

Statistical processing of materials was carried out using the "Statistica 10.0" application program package.

This prospective open clinical study was conducted at Uzhhorod National University from December 2022 to February 2023. Each patient involved in it signed an informed agreement for examination and treatment. This study was conducted in accordance with ethical principles in medicine. It is part of the scientific topic of the therapy and family medicine department of the faculty of postgraduate education (Uzhhorod National University) 36A-2021 "Innovative methods of diagnosis and treatment of internal organs pathology in obese patients", state registration number 0121U111773.

RESULTS

All 110 patients (mean age 51.3±4.8 years, 65 (59.1%) men) denied drinking alcohol and had negative laboratory test results for viral hepatitis B and C. In all 110 people included in the study, ultrasound signs of liver steatosis were detected. Diagnosis of MAFLD was detected according to CAP more than 2.2 dB/cm. It was steatosis of liver that was the criterion for the inclusion of patients in this study. Stage S1 was diagnosed in 42 (38.2%) patients with MAFLD, stage S2 was detected in 56 (50.9%) examined persons. Stage S3 was diagnosed only in 12 (10.9%) MAFLD patients. 72 patients (65.5%) were overweight or obese, but the morbid obesity (BMI≥40) was determined in only three (2.73%) of them. The carbohydrate metabolism disorders were found in 62 persons (56.4%). 38 (34.5%) patients among them suffered from type 2 diabetes. In 24 patients (21.8%) the compensated diabetes was detected. 14 (12.7%) persons suffered from the subcompensated T2DM. There was no decompensated diabetes among the examined. The lipid metabolism disorders were detected in the vast majority of patients included in this study. There was the presence of atherogenic dyslipidemia in the vast majority of patients

with MAFLD. Their lipid profiles were characterized by an isolated decrease of HDL level (14 persons, 12.7%), an isolated increase of LDL level (17 patients, 15.5%), a combined disorder (decrease in HDL + increase in LDL, 67 patients, 60.9%), hypertriglyceridemia (34 patients, 30.9%) and increased atherogenicity ratio (82 persons, 74.5%)

MAFLD in the stage of steatohepatitis (nonalcoholic steatohepatitis, NAFLD) was verified in 42 examined patients (38.2%). The other 68 persons (61.8%) had MAFLD in the stage of steatosis, without inflammation signs. The vast majority of cases of steatohepatitis (32 out of 42, 76.2%) was with minimal activity of the inflammatory process, as their ALT level did not exceed 3 norms.

The level of fecal calprotectin was examined (quantitatively) in all 110 patients with MAFLD. It was found that only 18 of all the examined had this indicator within the normal range. The minimal excess of FC (no more than 3 norms) was detected in 72 MAFLD patients (65.5%). The moderate increase of this indicator was found in another 12 MAFLD patients, in which fecal calprotectin level was more than 3 norms, but did not exceed a 10-fold increase. The fecal calprotectin level more than 10-fold excess of the norm was observed in only 8 MAFLD patients.

There were detected that independent predictors of steatosis were older age (adjusted odds ratio [aOR], 1.43; 95% confidence interval [CI], 1.13-1.78), higher BMI (aOR, 1.30; 95% CI, 1.19-1.40) and higher triglycerides (aOR, 1.46; 95% CI, 1.10-2.19). Patients who were classified as S2-S3 were older ($p < 0.01$) and were more often male ($p < 0.01$) than the persons with S1. In addition, participants with S2-S3 more frequently had metabolic syndrome, a history of diabetes ($p < 0.01$) and more frequently used antihypertensive, antidiabetic, and lipid-lowering drugs (all $p < 0.01$). BMI was higher in patients with a S2.

Fecal calprotectin levels were significantly elevated in MAFLD patients with S2-S3 compared to those with S1 (75.8 [42.9–112.1] *vs.* 46.3 [28.2–65.4], $p < 0.01$). The increasing age in MAFLD patients was not associated with a significant change of the level of fecal calprotectin.

Conducting two-dimensional shear wave elastography made it possible to detect fibrosis in 52 (47.3%) of the examined patients with MAFLD. In 37 (33.6%) of them, fibrosis was detected in stage F1, and in the remaining 15 (13.6%) patients, fibrosis was detected in stage F2. F3 was not detected among examined MAFLD patients. No signs of fibrosis were detected (stage F0) in 58 patients included in this study.

DISCUSSION

This study demonstrated that fecal calprotectin levels are increased in persons with MAFLD. The diagnosis of MAFLD in the patients included in this study was based on the results of ultrasound investigation by two-dimensional shear-wave elastography (2D-SWE) with associated controlled attenuation parameter (elastography and steatometry of the liver). Statistical analyses showed that fecal calprotectin levels were independently associated with steatosis stages after adjustment for relevant confounding factors, including comorbidities (diabetes, hypertension), cholesterol levels, insulin resistance. Statistical analyses demonstrated that there were significantly differential associations of calprotectin levels by BMI. Our results indicate that level of fecal calprotectin may be a promising biomarker for the presence of liver steatosis and the development of MAFLD.

We proved that the level of fecal calprotectin can reflect the involvement of neutrophils in the pathogenesis of MAFLD, similar to the results [12] regarding the level of calprotectin in the blood. Hepatic neutrophil infiltration is a salient feature of MAFLD progression, and several mechanisms have been proposed to explain how these neutrophils may accelerate disease progression [12]. We obtained convincing data on the association of fecal calprotectin only with the severity of steatosis, in contrast to other works [11], which described the correlation between inflammatory cytokines and the severity of fibrosis in the liver. The inflammatory cytokines inflict damage to hepatocytes and perpetuate inflammation and fibrosis [11]. Although the exact contribution of calprotectin to MAFLD pathogenesis is not well understood, serum levels of S100A8/S100A9 play a critical role in modulating the inflammatory response [5, 13]. Similarly, S100A8/S100A9 proteins are upregulated in experimental animal models of NAFLD, as well as in adipose tissue of patients with NAFLD [14]. Considering the role of calprotectin in other inflammatory diseases, it has been suggested that it serves a prominent role in innate immunity in the context of MAFLD-associated gut microbial dysbiosis [15]. In the present study, there were relations for the association between fecal calprotectin levels and steatosis stages. We cannot definitely explain these findings, but it seems that fecal calprotectin as inflammation biomarker may be a marker not only of the early pathogenetic MAFLD stages, but also the metabolic syndrome. Similar data were obtained [7] regarding the plasma level of calprotectin. Calprotectin is also deeply involved in the development of atherosclerosis mainly via the inflammatory process [16].

Taking into consideration the small group of patients with MAFLD involved in this study (only 110 people) and its short duration (only 3 months), the obtained results may probably not be transferred to the general population without additional research yet. However, the clinical significance of the data obtained in this study undoubtedly deserves attention and research in additional studies.

CONCLUSIONS

In conclusion, fecal calprotectin levels are significantly elevated in patients with MAFLD. Future studies are warranted to establish the definitive role and clinical utility of fecal calprotectin as a potential biomarker of probably liver steatosis as well as other diseases associated with methabolic syndrome and its complications.

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ANALYSIS OF THE BLOOD HYPERCOAGULATION RISK IN PATIENTS WITH ISCHEMIC ATHEROTHROMBOTIC STROKE DEPENDING OF THE VDR GENE POLYMORPHISMS

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ABSTRACT

Aim: of our study was the analysis of the blood hypercoagulation risk in patients with ischemic atherotrombotic stroke depending of the VDR gene polymorphisms.

Materials and Methods: Blood of 170 patients with ischemic atherothrombotic stroke (IATS) and 124 healthy individuals (control group) was used for genotyping. Four polymorphisms (FokI, BsmI, ApaI, TaqI) of gene VDR were examined with PCR-RFLP methodology. Statistical analysis was performed by using SPSS-17.0 program.

Results: Among patients with IATS who are carriers of the f/f genotype, FokI polymorphism of VDR gene by high thrombin time and a decrease in the rate of spontaneous fibrinolysis was registered. In individuals with the B/B genotype homozygous for the polymorphic variant, BsmI had significantly lower mean values of prothrombin and thrombin time and increased the rate of spontaneous fibrinolysis. The homozygotes for the A-allele ApaI polymorphism have 2.7 times higher risk of developing blood hypercoagulation than homozygotes for the a-allele was found.

Conclusions: Biochemical signs of hypercoagulation syndrome among patients with IATS who are carriers of the f/f genotype of the FokI polymorphic variant and among B/B homozygotes of the BsmI polymorphic variant and homozygotes for the A-allele of the ApaI polymorphism of the VDR gene were registered.

KEY WORDS: blood coagulation, ischemic atherothrombotic stroke, gene polymorphism, VDR

INTRODUCTION

The ischemic atherothrombotic stroke (IATS) accounts for 50% of cases and is the cause of disability in the middle-aged working population. Vascular disorders can be associated with changes in the structure of many genes. Given the presence of a hereditary component in the etiology of this disease, global scientific research is focused on identifying candidate genes, the products of which can lead to dysfunction of the vascular endothelium and disruption of the hemostasis system [1, 2, 3].

Among the main risk factors for complications of the atherosclerotic process, the factors that determine the hypercoagulable activity of the blood occupy an important place. Thus, an increase in the level of plasma fibrinogen is considered an independent factor that increases the risk of exacerbation of coronary heart disease by 3-4 times [4, 5, 6]. The vitamin D receptor (VDR) gene serves as a good candidate gene for susceptibility to essential hypertension. The gene regulates the renin angiotensin system by influencing blood pressure regulation. Around 3% of the human genome is regulated by the vitamin D endocrine system. Several studies are known to have reported mixed results regarding the relationship between the VDR gene and hypertension [7].

Calcifications of cerebral arteries are an unfavorable prognostic factor for the occurrence of fatal events stroke complications. One of the central links in the protection of blood vessels from ectopic calcification is the matrix protein Gla-protein (MGP), the presence of which in tissues prevents both the initiation of pathological calcification and its spreading Today, the factors involved in the regulation of MGP gene expression and possible mechanisms through which the anticalcinogenic properties of the protein are realized. This gives reason to talk about the MGP functional system, to which, in addition to the protein itself, may include such factors as the vitamin D receptor (VDR), enzymes that take participation in biochemical transformations of MGP, – vitamin K-oxidoreductase (VKOR) and γ-glutamylcarboxylase (GGCX), – and also possible targets for MGP, particularly bone morphogenetic protein 2 (BMP-2). The effective operation of these systems depends on many factors, one of which is the polymorphism of the genes encoding the structure of the corresponding proteins. A number of generally accepted indicators, including prothrombin time and prothrombin index, thrombin time, fibrinogen content in blood plasma, and the intensity of spontaneous fibrinolysis, assessed the state of blood coagulation processes in patients with IATS [8, 9].

AIM

The aim of our study was the analysis of the blood hypercoagulation risk in patients with ischemic atherothrombotic stroke depending of the VDR gene polymorphisms.

MATERIALS AND METHODS

SUBJECTS

The studied group included 170 patients with IATS (42.4% women and 57.6% men) aged 40 to 85 years (mean age 64.7 ± 0.73 years). The ischemic nature of the stroke according to the anamnesis and clinical picture of the disease, the results of an MRI study of the brain was established. The pathogenic variant of stroke was determined according to TOAST criteria [10], based on the anamnesis and features of the clinical course of the disease, ultrasound Doppler ultrasound, ECG. The control group consisted of 124 patients in whom the absence of cardiovascular pathology was confirmed by anamnestic data collection, electrocardiogram withdrawal and blood pressure measurements. The control group and the group of patients with IATS did not differ in the ratio of persons of different sex (P = 0.294 on the $\chi 2$ criterion), but the mean age of the first (76.7 ± 0.93 years) was significantly higher than the second was (P <0.001) [11].

AMPLIFICATION AND GENOTYPING

As described in our earlier research DNA for genotyping from the venous blood using commercially available kits (Isogene Lab Ltd) according to the manufacturer's protocol was extracted. Determination of polymorphisms *Fok*I- (rs 2228570), *Bsm*I- (rs1544410), *Apa*I- (rs7975232), and *Taq*I- (rs731236) of the *VDR* gene using the polymerase chain reaction method followed by restriction fragment length analysis upon detection by agarose gel electrophoresis was performed. Primers synthesized by Metabion (Germany) and enzymes (Taq polymerase and restrictase) by Thermo Scientific (USA) were used. PCR in a GeneAmp PCR thermocycler System 2700 ("Applied Biosystems", USA) was performed [12].

Detection of the restriction products by horizontal electrophoresis in 2.5% agarose gel (Sigma-Aldrich, USA) was performed containing ethidium bromide (Sigma-Aldrich, USA). The results in ultraviolet rays using an automatic video reading system "Vi-Tran" in a transilluminator ("Biocom") were visualized [11, 12].

STATISTICAL ANALYSIS

We performed statistical analysis using the SPSS-17 program. Before testing the statistical hypotheses, an analysis of the normality of the distribution of values in the samples was carried out, by determining the asymmetry and excess coefficients using the Willkie-Khan-Shapiro and Lilliefors criteria using the algorithms implemented in SPSS-17. The significance of the differences between the two samples was determined using Student's t test (t). Based on the magnitude of t and the number of degrees of freedom (l = n1 + n2-2), the difference between the two samples (P) was found on the Student's distribution table. The difference was considered significant if the probability of a random difference did not exceed 0.05 ($p < 0.05$). Non-parametric criteria to estimate differences in mean trends and independent samples were used, namely the Fisher exact method for a four-field table (TMF). The use of nonparametric criteria made it possible to find out significant differences in cases where the criterion t did not reveal them [12].

RESULTS

A number of commonly accepted indicators, including prothrombin time and prothrombin index, thrombin time, fibrinogen content in blood plasma, and the intensity of spontaneous fibrinolysis, assessed the state of blood coagulation processes in patients with IATS. In *Fok*I polymorphism homozygotes for the f-allele (f/f) the average value of thrombin time was slightly higher (P=0.047), and spontaneous fibrinolysis was lower (P=0.046) than individuals with genotypes (*F/F* and *F/f*). Other indicators did not depend on the genetic factor that was the subject of our analysis and were not statistically significant (P>0.05) (Table 1).

When the *Bsm*I polymorphism is studied, that homozygotes for the *B*-allele are characterized by lower average values of prothrombin (P=0,045) and thrombin time (P=0,048) and greater spontaneous fibrinolysis in the blood plasma (P=0.036), that is, they have biochemical signs of hypercoagulation syndrome (Table 1).

Data on blood clotting indicators in patients with IATS depending on their genotype according to the *Apa*I polymorphism of the *VDR* gene are also given (Table 1). It can be seen that homozygotes for the *A*-allele are characterized by lower average values of prothrombin time (P=0.020) and prothrombin index in blood plasma (P=0.011), that is, they have biochemical signs of hypercoagulation syndrome. And a data on blood coagulation parameters in patients with IATS depending on their genotype according to the *Taq*I polymorphism were also studied. It can be seen in Table 1 that the *Taq*I genotype does not affect the studied indicators.

The division of patients with IATS into two subgroups according to the presence and absence of functional and biochemical signs of blood hypercoagulation (patients with a prothrombin time < 9 seconds and a prothrombin index < 80% were considered prone to accelerated blood clotting) did not reveal any influence of the studied genetic a marker for the risk of developing hypercoagulable syndrome. Patients with a normal level of coagulation according to the *F/F* genotype were 23.1%, *F/f* genotype – 60.4% and *f/f* genotype – 16.5%. Among patients with IATS who have hypercoagulation syndrome, carriers of such genotypes were 24.1%, 45.6%, and 30.4%, respectively. Therefore, the distribution of allelic variants of the *VDR* gene according to the *Fok*I polymorphism is not significantly different in patients with IATI with a normal level of coagulation and hypercoagulation syndrome ($\chi 2=5.323$; P=0.007).

*Bsm*I polymorphism in patients with IATS was not associated with this syndrome. Thus, among patients with a normal level of coagulation, there were 49.4% with the

Table 1. Blood coagulation indicators in patients with IATS depending on the variants of the genotype according VDR gene polymorphisms (M±m)

	Prothrombin time, sec	Prothrombin index, %	Thrombin time, sec	Fibrinogen, g/L	Spontaneous fibrinolysis, min
			***Fok*I polymorphism**		
F/F (n=40)	9.26±0.27	82.6±2.07	16.4±0.59	3.92±0.20	484.6±6.5
F/f (n=91)	9.32±0.22	83.0±1.64	16.1±0.33	4.04±0.13	482.4±3.6
f/f (n =39)	10.05±0.34	88.3±2.17	17.7±0.65	3.70±0.18	466.8±5.5
F	2.088	2.108	3.107	1.056	3.135
P	0.127	0.125	0.047	0.350	0.046
			***Bsm*I polymorphism**		
b/b (n=71)	9.84±0.25	86.47±1.88	17.1±0.45	3.78±0.15	471.1±4.0
b/B (n=74)	9.39±0.23	83.51±1.63	16.6±0.41	3.91±0.13	483.9±4.2
B/B (n=25)	8.69±0.35	79.32±2.45	15.0±0.48	4.41±0.25	489.2±8.3
F	3.157	2.332	3.103	2.529	3.393
P	0.045	0.100	0.048	0.083	0.036
			***Apa*I polymorphism**		
a/a (n=45)	9.92±0.33	86.6±2.53	16.8±0.50	3.82±0.17	474.8±4.68
a/A (n=85)	9.58±0.22	85.6±1.57	16.9±0.42	3.82±0.14	476.9±3.92
A/A (n=40)	8.74±0.27	78.1±1.68	15.4±0.44	4.29±0.18	489.5±6.4
F	3.997	4.608	2.583	2.366	2.129
P	0.020	0.011	0.079	0.097	0.122
			***Taq*I polymorphism**		
T/T (n=68)	9.67±0.26	85.5±1.99	16.8±0.45	3.82±0.15	473.8±4.18
T/t (n=82)	9.49±0.22	84.5±1.51	16.6±0.38	3.90±0.13	480.2±3.98
t/t (n=20)	8.77±0.40	78.1±2.57	15.4±0.76	4.46±0.28	494.5±9.12
F	1.521	2.061	1.220	2.230	2.591
P	0.222	0.131	0.298	0.111	0.078

Note: n – the number of patients

b/b genotype, 40.5% with the *b/B* genotype, and 10.1% with the *B/B* genotype. Among patients with IATS who have hypercoagulation syndrome, carriers of such genotypes were 35.2%, 46.1%, and 18.7%, respectively. Thus, the distribution of allelic variants of the *VDR* gene according to the *Bsm*I polymorphism is not significantly different in patients with IATS with a normal level of coagulation and hypercoagulation syndrome ($\chi 2=4.457$; P=0.108).

It should be noted that the differences in the relationship between polymorphic variants of the VDR gene and the development of blood hypercoagulation are very close to the level of statistical significance. Thus, the ratio of genotypes a/a, a/A and A/A in patients without signs of blood hypercoagulation was 30.4%, 54.4% and 15.2%, while in patients with reduced indicators of prothrombin time and prothrombin index – 32.1%, 46.2% and 30.8% ($\chi 2 =5.794$, P1=0.055).

According to the data of the conducted regression analysis on the dependence between the *Apa*I polymorphism and blood hypercoagulation in patients with IATS, it was found that homozygotes for the *A*-allele have 2.7 times higher risk of developing blood hypercoagulation than homozygotes for the a-allele (Table 2).

The *Taq*I polymorphism in patients with IATS was not associated with this syndrome. So, among patients with a normal level of coagulation, there were with the *T/T* genotype 43.0%, *T/t* genotype 49.4%, and *t/t* genotype 7.6%. Among patients with IATS who have hypercoagulation syndrome,

Table 2. Analysis of the blood hypercoagulation risk in patients with IATS depending on the genotype by ApaI polymorphism of the VDR gene (logistic regression method)

	Genotype	CR	SE	WS	P	OR	95% CI for OR lower	95% CI for OR uppper
Blood hypercoagulation	a/A	0,110	0,369	0,089	0,766	1,116	0,541	2,302
	A/A	0,981	0,456	4,618	0,032	2,667	1,090	6,524

Note: A-allele homozygotes (A/A) are compared with a-allele carriers (a/A+a/a). CR – regression coefficient, SE – standard error, WS – Wald statistic, P – statistical significance, OR – risk ratio, CI – confidence interval.

carriers of such genotypes were 37.4%, 47.3%, and 15.4%, respectively. Thus, the distribution of allelic variants of the *VDR* gene according to the *Taq*I polymorphism is not significantly different in patients with IATS with a normal level of coagulation and hypercoagulation syndrome ($\chi 2 = 2.561$; $P = 0.278$) (Table 1).

DISCUSSIONS

During the study of blood coagulation indicators in patients with cerebrovascular pathology, biochemical signs of hypercoagulable syndrome characterized by a higher content of fibrinogen and lower average values of prothrombin time and prothrombin index were found in homozygotes for the B-allele of the *Bsm*I polymorphism and homozygotes for the A-allele of the *Apa*I polymorphism of the VDR gene.

The research of other scientists on this issue is ambiguous. Thus, Watzka et al. found no association between polymorphic variants of the *VKORC1* and *GGCX* genes and the level of vitamin K-dependent coagulation factors in the German population [13]. Marieke et al., examining patients with deep vein thrombosis, did not find an association between haplotypes *VKORC1*, *NQO1* and *GGCX* (polymorphisms rs6738645, rs699664, rs10179904, rs11676382, rs17026447, rs2028898) and the risk of venous thrombosis. Only for the H1 haplotype of the *GGCX* gene, according to the specified polymorphisms, a connection with a decrease in the activity of the blood coagulation factor II was found [14]. Vanakker et al. showed that the genetic polymorphism of the 8th exon Arg325Gln of the GGCX gene reduces carboxylase activity and induces deficiency of vitamin K-dependent blood coagulation factors. Scientists concluded that genetic variability of GGCX is a risk factor for severe neonatal bleeding [15]. Kimura et al. studied the effect of polymorphisms of GGCX, VKORC1 and CALU genes on the activity of C- and S-proteins among Japanese people. Women who were homozygotes for the major allele (Arg325Gln polymorphism of the GGCX gene) had significantly higher levels of protein C activity than heterozygotes and homozygotes for the minor allele [16]. When dividing patients into groups based on the presence and absence of blood hypercoagulation syndrome, we did not establish any relationship between the studied polymorphisms of the MGP system genes and the development of blood hypercoagulation syndrome in patients with ACS. Only in some groups of patients such an association was still found: in homozygotes for the minor allele (A/A) for the *G-7A* polymorphism of *MGP* gene with BMI≥25 kg/m^2, without obesity and with diabetes, and in f/f homozygotes for the *Fok*I polymorphism of the *VDR* gene with atherogenic dyslipoproteinemia and without diabetes, the hypercoagulation syndrome occurs more often.

CONCLUSIONS

Biochemical signs of hypercoagulation syndrome among patients with IATS who are carriers of the f/f genotype of the *Fok*I polymorphic variant and among B/B homozygotes of the *Bsm*I polymorphic variant of the *VDR* gene were registered. In A/A homozygotes of the *Apa*I polymorphic variant who have a stroke, the risk of hypercoagulable blood changes is 2.7 times higher than in patients with the a/a genotype.

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PROSPECTIVE SINGLE CENTER ANALYSIS OF OUTCOME STEM CELLS TRANSPLANTS IN PATIENTS WITH CEREBRAL PALSY

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ABSTRACT

Aim: To evaluate efficacy and safety of autologous bone marrow-derived mononuclear stem cell transplantation intrathecal in children with cerebral palsy.

Materials and Methods: 35 children have levels I-V cerebral palsy aged 8-months to 8-years-old were enrolled from September (2021-2022) at Iraqi private hospital. Gross Motor Function was assessed by a pediatrician and neurologist specialist, 5 mcg/kg/day of G-CSF subcutaneous single injection daily for three consecutive days. Bone marrow harvested from posterior iliac crest under light general anesthesia. Bone marrow mononuclear cells (BMMNCs) separation was performed using density gradient centrifugation with Ficoll, the cell viability checked by propidium iodide dye in a TALI machine (Invitrogen) in average 98%. The viable BMMNCs injected intrathecal in L4–L5 over a period of 5–10 min.

Results: Males accounted for 57.14% (20/35) while female 42.86% (15/35), and main neurological symptoms included spastic disorder spastic disorder (quadriplegia 24 (68.6), tetraplegia 2 (5.7), diplegia 5 (14.28), hemiplegia4 (11.42)). Gross Motor Function Classification System and Gross Motor Function Measure-66 (GMFM-66) showed II 10 (28.58), III 11(31.42) and IV 14 (40). On mean follow-up of 3 months post-stem cell transplant improvement was observed in 80% cases. The improvement showed in gross motor function (6/8) p=0.01, and speech (2/4) p=0.04, neck holding (5/5) p=0.0003, sitting balance (4/4) p=0.04, postural tone (5/5) p=0.0003, as well as significant reduction in seizure frequency (2/3) p=0.04 and improvement in cognition (6/7) p=0.01 were observed.

Conclusion: Stem cell therapy for cerebral palsy shows a significant positive effect on the gross motor function, without long adverse effects.

KEY WORDS: cerebral palsy, stem cells transplants, bone marrow aspiration, GMFC

INTRODUCTION

Cerebral palsy (CP) is a heterogeneous group of motor impairments caused by nonprogressive insults to the fetal or neonatal brain [1]. In the USA alone, approximately 10 000 babies and infants are diagnosed with cerebral palsy each year, making it the most common motor disorder of childhood and worldwide prevalence is constant at 2-3 per 1000 newborns for more than four decades, despite substantial improvements in perinatal medicine [2]. Cerebral palsy produces chronic motor disability in children and the causes are quite varied and range from abnormalities of brain development to birth-related injuries to postnatal brain injuries [3]. While premature infants and term infants who have suffered neonatal hypoxic-ischemic injury represent only a minority of the total cerebral palsy population [4]. The cause of CP remains elusive, and the precipitating factors include prematurity, low birth weight, birth asphyxia, fetal intrauterine inflammation and infection, hypoxic and ischemic events of fetus, genomic abnormalities, and many uncertain [5]. Stem cell transplantation promising treatment for cerebral palsy and still in the initial stages of investigation with many novel results in clinical trials [6]. Several types of stem cells are candidates for the treatment of cerebral palsy, such as human embryonic neural stem cells, olfactory unsheathing cells, umbilical mesenchymal stem cells (MSCs), and bone marrow MSCs [7]. Theses stem

cells has ability to differentiate into few (like multipotent adult stem cells) or any (like pluripotent embryonic stem cells) cell types while able to self-renew to produce more stem cells [1]. Scientific investigators have been working to find and recognize the molecular and cellular pathways that involve stem cells [8]. Deep understanding of the pathophysiological mechanisms related to stem cells will be the only way to obtain them in a laboratory and use the achieved results in the treatment of neurological disorders like cerebral palsy [9]. Hematopoietic stem cells type of stem cell has been used in various clinical trials for the treatment of CP [10]. The mononuclear stem cells are hematopoietic cells obtained from bone marrow defined as CD 34+ cells that have the ability of self-renewal, differentiation, and migration [11]. In the human clinical trials on cerebral palsy children, there has been an acceptable reduction of disability scale after the introduced of stem cells derived from the bone marrow into the systemic circulation [12]. One of the main ideas inherent in stem cell transplantation for cerebral palsy is that the stem cells would replace the cells of the damaged nervous system. Most reports dealing with adult stem cells show only a minimal survival of the transplanted cells with few, if any, of these cells displaying markers/functionality of nervous tissue [1]. It does not appear that replacement alone would be sufficient to account for improvement in the experimental situation [7]. While embryonic or iPS cells may have somewhat greater potential for such replacement and transformation, the number of cells undergoing this process is quite limited in vivo [13]. Even though there may be some replacement by transplanted cells, the cells often do not develop normal processes and may not function in neuronal circuitry [14]. Thus, cell replacement as an explanation for any improvement in the models is unlikely to be the case given the current state of our knowledge of the cell biology of stem cells.

AIM

The primary aim of this study is to evaluate the efficacy and safety of autologous bone marrow-derived mononuclear stem cell transplantation intrathecal in combination with rehabilitation in children with cerebral palsy.

MATERIALS AND METHODS

STUDY DESIGN AND PATIENTS

The procedures followed the ethical standards described by the Helsinki Declaration and carried out in accordance with the local and national regulatory guidelines [15]. A written informed consent was obtained from the adult patients and parents of all the children. The intervention was explained to them in detail along with possible adverse events. Thirty-five children have levels (II -V) of cerebral palsy aged 8 months to 8 years were enrolled in this study from September 2021 to September 2022 at Iraqi private hospital. The Gross Motor Function Classification System (GMFCS, corresponding to GMFCS E&R 2007) was assessed by a pediatrician and neurologist specialist. Children with autism, active systemic infection, treatment with chemotherapy, epilepsy, or seizures, severe anaphylactic or autoimmune diseases, failure of the heart, lungs, liver, kidneys, and possible brain malformations or genetic conditions were excluded from this study. Routine blood tests, and assess the presence of virus markers for HIV, hepatitis B, and C, X-rays, serum creatine phosphokinase (CPK), Imaging studies of the brain, spine, and musculoskeletal system were performed.

HARVESTING BONE MARROW AND STEM CELL COLLECTION

The patients received granulocyte colony stimulating factor G-CSF (Neupogen, filgrastim 300 mcg) 5 mcg/kg/day administered subcutaneous injection single dose daily for three consecutive days in order to stimulate their bone marrow to produce stem cells. Bone marrow harvested from posterior iliac crest under light general anesthesia in operation room after 24 hours from last G-CSF injection using bone marrow aspiration needle and collected in heparinized tubes as we described in our previous publication [15]. Bone marrow mononuclear cells (BMMNCs) separation was performed using density gradient centrifugation with Ficoll, and the cell viability was checked by propidium iodide dye in a TALI machine (Invitrogen) in an average 98%. The CD34+ counting was done by Fluorescence Activated Cell Sorting (FACS) using CD34 PE antibody (BD Biosciences) [16]. The viable (BMMNCs) number of cells was an average 10.12×10^6 injected immediately intrathecal in L4–L5, via lumbar puncture using fine-gauge spinal needles (Touhy needle) in sizes ranging from 18-gauge to 29-gauge under aseptic procedure over a period of 5–10 min. All children monitored for any procedure associated with adverse events (Fig. 1A-B.).

NEUROLOGICAL ASSESSMENT

GMFM-66 and Peabody Motor Quotient Gross and Fine scores were used to assess the changes in neurological sequelae before and after stem cells transplant [17].

ELECTRO-ENCEPHALOGRAPHY

Electro-encephalography (EEG) was done in patients with cerebral palsy to assess the electrical activity in the brain especially in children with seizures. The pattern of activity in the brain is specific, and when seizures occur, there are clear deviations from normal activity. EEG was carried out as a tool to pick up the abnormal brain electrical activity and also to compare postoperatively in patients whom the parents had reported a reduction in seizure frequency posttherapy.

IMAGING ASSESSMENT

Before the treatment, all the patients underwent magnetic resonance imaging (MRI) scans for the brain. The MRI was also carried out on follow-up to compare the changes.

ETHICAL APPROVAL

Ethical approval to report this case series was obtained from Iraqi Private Hospital ethics committee's approval number/2N/M2023.

All procedures in this study were conducted in accordance with our IRB at Iraqi Private Hospital ethics committee's approval identification protocol/ 003Yousif et al/2023.

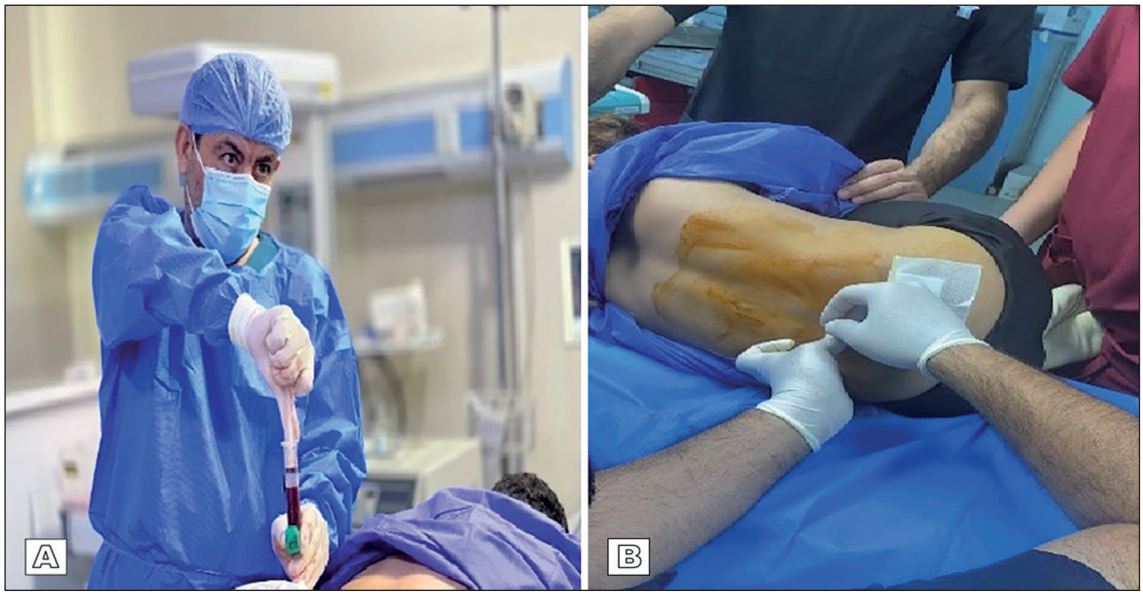

Fig. 1. Harvesting bone marrow from posterior ilaic creast **(A)**. Injection of stem cells intrathecal **(B).**

Table 1. Demographical data of cerebral palsy patients (n = 35).

Characteristics	No. (%)
Patient characteristics	
Sex	
Male	20 (57.14)
Female	15 (42.86)
Type of cerebral palsy	
Spastic quadriplegia	24 (68.6)
Spastic tetraplegia	2 (5.7)
Spastic diplegia	5 (14.28)
Spastic hemiplegia	4 (11.42)
GMFCS level	
II level	10 (28.58)
III level	11(31.42)
IV level	14 (40)

Written informed consent was obtained from the patient's parents for their anonymized information to be published in this article.

STATISTICAL ANALYSIS

The data were expressed as the mean ± S.E.M. Characteristics statistics were used to define the demographics of patients with CP. The statistical significance of the patient ages, GMFM scores and language developmental quotients was detected using the one-way ANOVA analysis, LSD test and two-tailed Student's t-test. The statistical significance of the GMFCS levels was detected using the chi-squared test. Data analyses were performed using SPSS 11.0 [18].

RESULTS

PATIENTS CHARACTERISTICS

Thirty-five children with cerebral palsy patients underwent autologous bone marrow-derived mononuclear (BMMNCs) cell transplantation with age ranged from 8 months to 8 years, mean age 4.3. Males accounted for 57.14% (20/35) while female 42.86% (15/35). The neurological symptoms included spastic disorder (quadriplegia 24 (68.6%), tetraplegia 2 (5.7%), diplegia 5 (14.28%), hemiplegia 4 (11.42%)), cognitive impairment, swallowing and speech problems. The Gross Motor Function Classification System (GMFCS, corresponding to GMFCS E&R 2007) and the total score of for all CP children range from II – IV levels showed that II level was 10 (28.58%), III level – 11 (31.42%) and IV level – 14 (40%). Gross Motor

Function Measure-66 (GMFM-66) with total score 37 (23-58) Minimal score 28.5 Maximum score 49 (Table 1).

FOLLOW UP MEASUREMENT

On mean follow-up of 3 months, post-stem cell transplantation improvement was observed in 80% cases (Fig. 2). The improvement showed in gross motor function (6/8) p=0.01, and speech (2/4) p=0.04, neck holding (5/5) p=0.0003, sitting balance (4/4) p=0.04, postural tone (5/5) p=0.0003, as well as significant reduction in seizure frequency (2/3) p=0.04 and improvement in cognition (6/7) p=0.01 were observed (Table 2).

MRI EVALUATION

Periventricular white matter injury (PWMI) was the most common pattern observed found in 66% scans mostly spastic diplegia (23/35). From 35 children who underwent MRI, 2 (5.71%) had normal MRI. Diffuse encephalopathy was found in 2% scans from spastic quadriplegia p=0.03. Focal ischemic lesions present in (1/35) p=0.09. Two children had encephalopathy reported on their MRI. Spastic diplegia (35/47). In children with hemiplegic CP (n=4/35) MRI finding showed Malformations p=0.8. One child had focal ischemic or hemorrhagic lesions found in spastic diplegia (n=5/35) p=0.09 (Table 3).

OVERALL IMPROVEMENTS

Overall improvements after stem cell therapy in cerebral palsy (CP) patients (total 35), 7/35 showed no improvement, 2/35 with mild improvement, 7/35 moderate improvement and 19/35 significant improvement p=0.005 (Fig. 2).

MOTOR EVALUATIONS

More evaluation was done through motor assessments after three months duration followed cell therapy. GMFM-66 showed that children with CP improved by 3.4 ± 1.3 (95% CI, 3.4-6.2), Peabody Motor Quotient Gross and Fine (PDMS, Peabody Developmental Motor Scales) 0.7 ± 3.3 (95% CI, 0.9-3.2) and 0.2± 4.5 (95% CI, 2.3–3.1) respectively (Table 4).

All 35 children underwent EEG exams, while abnormal results were shown in 14 patients at baseline. Among the 5 children with slowing of EEG basic rhythms in EEG reports, less diffuse slow waves were noticed in all 5 patients after stem cells transplant with improved EEG status.

DISCUSSION

The mechanism of action by which stem cells may improve motor function in children with CP is not completely understood, it is well known that specific areas of brain are especially vulnerable to injury following hypoxic ischemic insult at certain periods [16]. The periventricular white matter is most susceptible before 32 weeks of gestation, whereas the subcortical white matter and basal ganglia are more prone to damage following hypoxic-ischemic encephalopathy in the last trimester of pregnancy [25, 26]. Language disorders

Table 2. Statistical analysis for each symptomatic improvement after stem cells transplants in patients with cerebral palsy

Symptoms	Chi-square	No. patients affected	No. patients improved	Value
Seizure frequency	20.2	3	2	0.04*
Cognition	20.2	6	7	0.01*
Neck holding	24.0	5	5	0.0003*
Sitting balance	19.0	4	4	0.04*
Postural tone	32.0	5	5	0.0003*
Gross motor function	27.4	8	6	0.01*
Speech	25.0	4	2	0.04*

**Significant at value ≤ 0.05*

Table 3. MRI pattern types among cerebral palsy subtypes in the study subjects (n = 35).

Spastic pattern MRI Categories	Spastic quadriplegia (n = 24/35)	Spastic tetraplegia (n = 2/35)	Spastic diplegia (n = 5/35)	Spastic hemiplegia (n = 4/35)	Total (n = 35)	P value
Normal MRI	3	0	1	1	5	0.6
Diffuse encephalopathy	2	0	0	0	2	0.03
Periventricular white matter injury	17	1	2	1	21	0.003
Focal ischemic or hemorrhagic lesions	0	0	1	0	1	0.09
Malformations	0	0	0	2	2	0.8
Unclassified findings	2	1	1	0	4	0.6

**Significant at value ≤ 0.05*

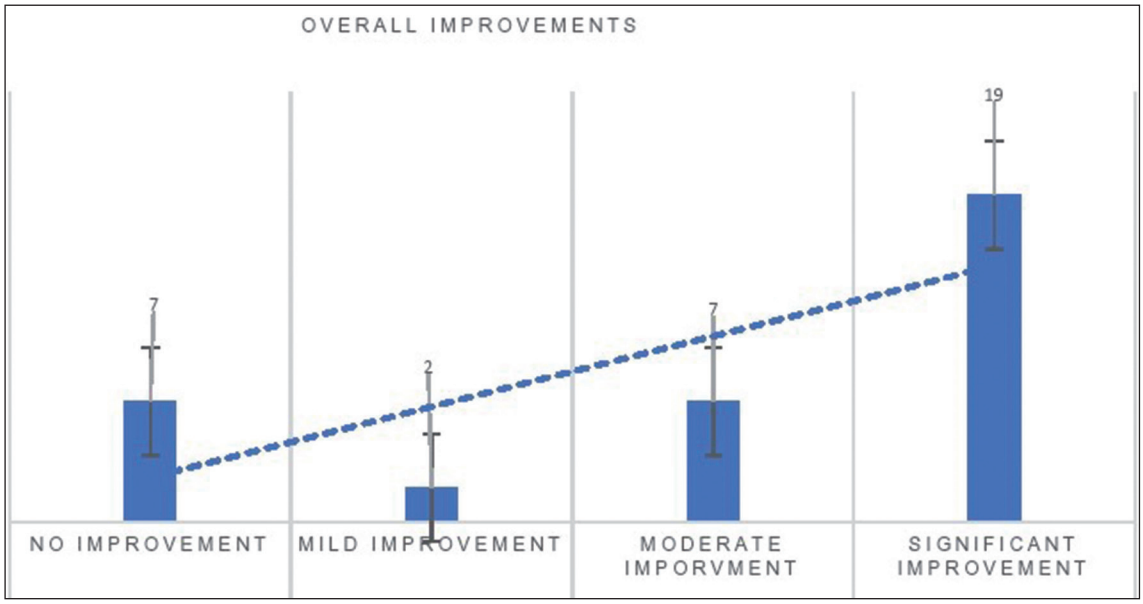

Fig. 2. Overall improvements after stem cell therapy in cerebral palsy (CP) patients.

Table 4. Motor assessments after three months duration followed cell therapy

Measures	Baseline, mean ± SD	3 months, mean ± SD	mean ± SD (95% CI)
GMFM 66	29.0±2.1	32.7±4.1	3.4±1.3 (2.6-7.1)
PDMS-Gross Motor Quotient	33.3±5.4	36.5±6.3	0.7±3.3 (0.9-3.2)
PDMS-Fine Motor Quotient	54.5±7.4	58.3±4.7	0.2±4.5 (2.3-3.1)

in children with cerebral palsy are categorized as anarthria and language developmental delays [17], which are caused by a motor disturbance of the speech organs due to brain injury [18]. It is well-known that stem cells have the ability to differentiate, and under appropriate conditions, become any type of cell in the human body. Stem cells also have the ability to proliferate indefinitely [19]. Stem cells have been applied in the clinical study of a variety of neurological diseases [20], and have shown good therapeutic effectiveness and safety for many diseases that cannot be medically or by surgical intervention. In this study, we found evidence of accelerated recovery of gross motor function in stem cells transplantation children with CP (P=0.01). Many published manuscripts have been reported in multiple clinical trials that bone marrow stem cell transplantation results in cognitive improvement [21], whereas others did not [22]. Almost all CP patients display muscle tone changes in clinical trials of stem cell therapy [23]. While these changes in muscle tone have not been reported in the therapy of stem cells derived from peripheral blood or umbilical cord [24]. Other resulted data reported that after 6-month follow-up after autologous BMMNCs transplantation, children with CP showed significant improvements in lying, rolling, and sitting, with a proportion of 98%, as well as improvements in walking, running, and jumping, with a proportion of 21% [25]. Also, this data is consistent with other outcomes in other clinical trials using stem cells derived from bone marrow [26]. This present study showed an overall improvement 80% from GMFM-66, Peabody Motor Quotient Gross, and Fine all motor evaluations. While only 20% not get benefits from stem cells transplant and without any adverse effects. However, in the present study, of the 35 children with cerebral palsy, periventricular white matter injury (PWMI) was the most common pattern observed found in 66%. This observation is similar to that seen in the other cohort study and mostly with Austrian group [27]. In our study, PWMI was most identified in spastic diplegia (23/35) and this finding supported by other research groups [28]. This is explained by the fact that the axons sub-serving the lower extremities are located closest to the ventricle and the axons of the upper extremities are situated lateral to them. In this study, the cerebral function might be improved since better cerebral electrophysiologic trace was observed in the EEG of some patients. However, cerebral structure improvements were not observed in routine MRI results as assessed previously [29]. This might be due to insufficient sensitivity of routine MRI to measure the changes at the cellular level. Diffusion tensor imaging (DTI) was superior to routine MRI on corticospinal tract (CST) imaging, and the correlation between DTI parameters of the injured CST and the severity of motor dysfunction had been confirmed in previous studies [30].

LIMITATIONS OF THIS STUDY

The main limitations of this study were that it did not have a placebo group to compare the outcome results and the duration of follow-up was short.

CONCLUSIONS

Our data indicates that the transplantation of stem cells is safe and effective for the treatment of chronic cerebral palsy. Motor function, but not the language quotient, indicated optimal improvement 3 months after transplantation. Basic knowledge of stem cell biology, satisfactory model of chronic injury, multi-center trials with homogenous patient groups. The problem remains difficult for several reasons: cerebral palsy is not a homogeneous disease, our knowledge of stem cell biology is in its infancy, the pre-clinical models are far from ideal, and various preclinical trials show efficacy in acute models leading to falsely raised hopes.

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MODERN TRENDS OF CHANGES IN THE MICROBIOTA OF UROGENITAL SYSTEM IN PATIENTS WITH UROLITHIASIS

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ABSTRACT

Aim: Analysis of trends in the microbial communities of the genitourinary system in patients with urolithiasis.

Materials and Methods: 165 urine samples from patients with urolithiasis was examined. The quantitative isolation of microflora was carried out using the bacteriological method. Microorganisms were identified using API biochemical test systems (bioMerieux, France). The percentage of the various types of microorganisms was determined. The reliability of differences in the frequency of various types of microorganisms isolation in a monoculture and the composition of microbial associations was determined by two-field tables with Fisher's Exact criterion. The GraphPad Prism 7 software was used.

Results: 198 bacterial cultures of various types were isolated. In one case, a *Candida* culture was isolated. *E. faecalis* was the most frequently isolated culture (29.1% of the isolated strains number); *E. coli* (18.1% of the total number of isolated cultures; *K. pneumoniae* (11.1%). There were no significant differences in the rate *of E. faecalis* compared to *E. coli*. *K. pneumonia* was isolated significantly less frequently than *E. faecalis*. These types of microorganisms were also leaders in the formation of bacterial mixes. In addition, these species are involved in the urease activity of bacteria (directly or indirectly), which contributes to the formation of stones in the genitourinary system.

Conclusions: *E. faecalis* is the species most often isolated from patients with purulent-inflammatory processes in patients with urolithiasis, both in the case of mono-infection and as part of mixed bacterial cultures.

KEY WORDS: *E. faecalis*, uropathogen, opportunistic infection, mixed infection

INTRODUCTION

Opportunistic infectious pathology is an urgent problem for modern medical science [1,2]. The pathogenicity factors of conditionally-pathogenic microorganisms are among the unsolved, actively studied questions [3], the mechanisms of their interaction with the affected cells and tissues [4]. In particular, for urolithiasis, the question of the microorganisms' contribution to the process of stone formation is essential [5]. The microbial in urolithiasis is currently in the process of active study and remains relevant. Moreover, being generally similar, the data presented by various authors have some differences. Maybe it is due to the influence on the study results of the characteristics of the examined groups of patients, nutrition, climatic conditions of people living in a particular country, level of medical care, primarily with the widespread use of antibiotics in medical practice.

AIM

Analysis of trends in the microbial communities of the genitourinary system in patients with urolithiasis.

MATERIALS AND METHODS

The studies were carried out in the bacteriological laboratory of Municipal Enterprise Poltava Regional Clinical Hospital, named after MV Sklifosovsky, Poltava, Ukraine, from 2020-to 2021. According to the Ethics Commission of Poltava State Medical University, the examination procedure for these individuals was conducted following the standards of the ethics committee. The material from patients was taken before the antibacterial therapy. The study was carried out of 165 samples of the middle portion of urine from patients diagnosed with urolithiasis. The bacteriological method was used following the normative and methodological recommendations [6]. The quantitative isolation of cultures was carried out according to the Gold method. In addition, the results in which the content of microbial cells was not less than 10^5 in 1 ml of urine was taken into account. The isolated microorganisms were identified using API biochemical test systems from bioMerieux (France).

The percentage ratio of various microorganisms from the number of isolated cultures and the number of investigated

Table 1. The frequency of selection of microorganisms from the urine taken from patients with urolithiasis

№	Groups, species	Total (number)	Sum (mono+mixes)	From the number of isolated strains (out of 199)		From among the studied samples (out of 165)	
				Total (%)	By groups (%)	Total (%)	By groups (%)
	Gram-positive bacteria						
	Staphylococci						
1	*S. aureus*	2/5	7	3,5		4,2	
	*S. epidermidis**	1/0	1	0,5		0,6	
2	*Coagulase-negative staphylococcus*	15/3	18	9,0	13,0	10,9	15,7
	Streptococci						
3	*S. agalactiae**	5/0	5	2,5		3,0	
4	*Bacteria of the genus Streptococcus*	9/0	9	4,5	7,0	5,5	8,5
	Enterococci						
5	*E. faecalis*	43/15	58	29,1		35,2	
6	*E. faecium*	8/3	11	5,5	34,6	6,7	41,9
	Other gram-positive bacteria						
7	*Corynebacterium spp.*	0/2	2	1,0	1,0	1,2	
	Total gram-positive bacteria				55,6	67,3	
	Gram-negative bacteria						
	Enterobacteria						
8	*E. coli*	28/8	36	18,1		21,8	
9	*K. pneumoniae*	10/12	22	11,1		13,4	
10	*P. mirabilis*	3/2	5	2,5		3,0	
11	*S. marcescens*	1/2	3	1,5		1,8	
12	*K. oxytoca*	1/1	2	1,0		1,2	
13	*Enterobacter**	2/0	2	1,0		1,2	
14	*Citrobacter*	1/1	2	1,0	36,2	1,2	36,9
	Non-fermenting bacteria						
15	*Acinetobacter*	6/3	9	4,5		5,5	
16	*P. aeruginosa**	2/0	2	1,0		1,2	
17	*Another non-fermenting bac.*	2/1	3	1,5	7,0	1,8	8,5
	Other gram-negative bacteria						
18	*H.parainfluenza***	0/1	1	0,5	0,5	0,6	
	Total				43,7	46,0	
	Fungi						
19	*Candida***	0/1	1	0,5	0,5	0,6	
	Total	139/60	199	99,8 (100)	99,8 (100)	100,0	

Notes: 1. Data are presented as fractions (X/Y), where the numerator represents the number of strains isolated in the monoculture and the denominator - from the mixes;
2. microorganisms were isolated only in monoculture;*
*3.** microorganisms were isolated only as part of mixed samples.*

samples was determined. The statistical reliability of differences in the frequency of isolation of microorganisms in monoculture and mixed cultures' composition was also determined. The frequencies of their obtaning were compared in two-field tables with the determining of Fisher's Exact criterion. The data were processed in absolute numbers. The GraphPad Prism 7 software was used.

RESULTS

Table 1 presents data of the frequency of various microorganisms isolation from the test material taken from patients with urolithiasis. 199 cultures of microorganisms were isolated in a bacteriological study of 165 urine samples taken from patients with urolithiasis (Table 1). 198 bacterial cultures and 1 culture of the *Candida* genus fungi were isolated. The predominant number of microorganisms belonged to gram-positive bacteria (staphylococci, streptococci and enterococci) and gram-negative bacteria (enterobacteria and gram-negative non-fermenting bacteria). In addition, in some cases, *Haemophilus parainfluenza* (1 case), *Corynebacterium* (2 cases) were isolated.

E. faecalis was isolated in monoculture in 43 cases and as part of mixed cultures – in 15 cases. 11 cultures of *E. faecium* isolates were also obtained (8 isolates in monoculture

and 3 in mixed cultures). Enterococci rate was 34.6% of all selected isolates. Moreover, it was exceeded the isolates of staphylococci (13%) and streptococci (7.0%) significantly.

Among the isolated gram-negative bacteria, the preponderate amount was represented by representatives of the *Enterobacteriaceae* family (36.2% of the total number of all isolated microorganisms). Half of this number were *E. coli*, isolated mainly in monoculture (28 cases). 8 straines were isolated from the mixed cultures. In total, *E. coli* accounted for 18.1% of the total number of isolated microorganisms.

K. pneumoniae was the following species on the frequency of occurrence (11.1%). In total, 22 isolates of bacteria of this species were isolated, of which 10 were in monoculture.

Thus, the dominant species turned out to be: *E. faecalis* were identified in 58 cases (29.1%), including 15 times in the mixes; *E. coli* (18.1%), *K. pneumoniae* (11.1%), coagulase-negative staphylococcus (9%), *E. faecium* (5.5%), bacteria of the genus *Streptococcus* and *Acinetobacter* (4.5% each), *S. aureus* (3.5%). In total, this amounted to 85.3%. The rest of the microorganisms accounted for 2.5% or less of the total cultures.

E. faecalis turned out to be the leader in participation in the mixed cultures (60.7%): in four cases with *E. coli*; 3 combinations each with *S. aureus*, *E. faecium* and *K. pneumoniae*. The remaining 4 cases are represented by single combinations of this microorganism with other species (in one of the cases, with *Candida* fungi). The second position was occupied by *K. pneumoniae* (9 cases). In addition to three mixes with *E. faecalis*, in two cases, combinations with *E. coli*, and in isolated cases with *S. aureus*, coagulase-negative staphylococci, *P. mirabilis* and *S. marcescens*. *E. coli* has been identified seven times in associations. In addition to four combinations with *E. faecalis*, two cases with *K. pneumoniae* and one case with *K. oxytoca*. *S. aureus* was the fourth most frequently involved in mix formation (5 cases). In 3 cases out of 5 identified, these were mixed with *E. faecalis*. In fifth position (4 cases each) were *E. faecium* and coagulase-negative staphylococci. Moreover, for coagulase-negative staphylococci, these were combinations with various microorganisms (*E. faecium, K. pneumoniae, S. marcescens, P. aeruginosa). E. faecium,* on the contrary, was found in three cases in combination with *E. faecalis* and only in one – with coagulase-negative staphylococcus.

When the mixed mixture consisted of 3 different microorganisms in all three cases, the microbial association included *E. faecalis* and *K. pneumoniae*. The variable portion was represented by *E. coli, Acinetobacter* and *Alcoligenes faecalis*.

DISCUSSION

Uropathogens are represented primarily by species that usually inhabit the human intestine. However, the microbiome excreted from the urine of patients with urolithiasis correlates to a greater extent with the microbiome isolated directly from stones and much less – with the species composition of microorganisms isolated from the urine of patients without urolithiasis [4]. Disruption of the genitourinary system microbiome, usually not accompanied by disorders of the intestinal microbiome [7]. In addition, the genitourinary microbiome, to a greater extent than the gut microbiome, is subject to changes due to antibiotic use [7].

The list of "classic" uropathogens is represented in the literature primarily by gram-negative bacteria – *E. coli* (45-80%), as well as *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*. A lesser role is given to gram-positive bacteria – bacteria of the genus *Staphylococcus*, including coagulase-negative species (*S. saprophyticus* and *S. epidermidis*), *Streptococcus* group *D, Enterococcus*. Among the anaerobic forms of bacteria, it is customary to call bacteroids. Today *Streptococcus agalactiae, Aerococcus, Citrobacter kosery, Enterobacter, Serratia*, capnophilic bacteria, *Actinomyces, Prevotella, Corynebacterium urealyticum*, *Gardnerella, Actinobacilum* (subclass *Actinomycetes*), *Candida albicans* are listed as potential uropathogens. The existing trend towards increasing the frequency of *E. faecalis* from patients with urolithiasis is associated with the widespread use of antibiotics in modern medical practice [10].

The data of this study essentially correspond to the generally accepted ideas about the species composition of microorganisms isolated from patients with urolithiasis. At the same time, as a result of our statistical analysis, for the first time, a significant quantitative predominance of *E. faecalis* over *E. coli,* which was in the second position in terms of the number of isolated strains was found ($P < 0.05$). It was especially true of *K. pneumoniae*, which occupies the third position in terms of the number of isolated cultures and other, less abundant species and groups of microorganisms.

The altered microbiome may directly contribute to the process of stone formation [8]. Particular importance is attached to the presence of urease activity of microorganisms secreted from patients with urolithiasis. It is believed that this enzyme is directly related to the formation of stones. However, urease activity is not always a property necessary to ensure the vital activity of producing bacteria; therefore, this property should be attributed to pathogenic factors capable of protecting microorganisms from the immune factors of the human body [5]. *E. faecalis* is not one of the bacteria producing urease, but it is characterized by associated with other urease-forming bacteria. It has been shown that in associations, *E. faecalis* has a potent effect, enhancing the manifestation of the pathogenic properties of other members of the association and promoting the formation of biofilms. The following two species, prevailing among cultures isolates from pathological material – *E. coli* and *K. pneumoniae* – may produce urease [10].

Thus, the examples of mixed infections identified in our study are consistent with the literature data. The analysis of the frequencies of isolation of microorganisms in a monoculture and the composition of microbial associations in two-field tables with the determining of Fisher's exact criterion was carried out for the cultures most often isolated from patients: *E. faecalis, E. coli* and *K. pneumoniae*. The statistical analysis results showed no significant differences in the propensity to form microbial

associations between *E. faecalis* and *E. coli* (P> 0.05). On the other hand, *K. pneumoniae*, which was isolated from the composition of associations more often than in monoculture, is significantly more (P <0.05) inclined to be present in the composition of associations than *E. faecalis* and *E. coli*. Such microorganisms and their combinations were found, contributing to the formation of stones in the genitourinary system. The same three species of bacteria were included in 5 species isolated from the composition of three-component associations found in six study cases. Moreover, in three out of six samples, both *E. faecalis, E. coli,* and *K. pneumoniae* were present. At the same time, some species were isolated only in monoculture (*S. epidermidis, S. agalactiae, Enterobacter, P. aeruginosa*), and others only in mixed-species (*Haemophilus parainfluenza, Corynebacterium*).

CONCLUSIONS

E. faecalis is the most numerous species isolated from patients with purulent-inflammatory processes in patients with urolithiasis. *E. coli* and *K. pneumoniae* occupy the second and third positions in the list of cultures isolated from pathological material taken from patients with urolithiasis. The indicated species are also leading in their participation in the bacterial mixes. It applies to both two-component and three-component microbial associations.

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FRUCTOSAMINE VALUES IN MONITORING COMPENSATION AND EFFICACY OF DIABETES MELLITUS TREATMENT

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ABSTRACT

Aim: The relevance of the study is determined by the objective of finding an optimal type of diagnostics of carbohydrate metabolism, that would assess the condition of a diabetic patient undergoing treatment. The purpose of the study is to create a model for monitoring the efficacy of diabetes mellitus treatment by determining the fructosamine levels.

Materials and Methods: The methods for investigating the highlighted issue are clinical examination and laboratory diagnosis of diabetic patients to measure the state of carbon metabolism using ion-exchange chromatography to determine glycated haemoglobin levels and an automatic colorimetric method to determine fructosamine levels.

Results: The study presents certain values of fructosamine over the level of changes in the state of patients with diabetes mellitus, reflecting the progress from the treatment in the compensation of carbohydrate metabolism, which allows creating a model of diagnostic values of the fructosamine levels, according to which the efficacy of treatment of diabetes mellitus, the state of progress of the disease in its compensation or decompensation are determined at a qualitative level.

Conclusions: This allows for the timely adaptive corrective therapeutic and preventive measures to be carried out by medical personnel, who, using values, will monitor the efficacy of treatment in each patient once every three weeks, as this will determine the influence of the type of conducted treatment or other factors aimed at compensating for pathogenetic and clinical manifestations of the disease, which makes the identified fructosamine criteria an important component in the treatment of diabetes mellitus, and indirectly allows to improve the life quality of this patient population, thus bringing a practical solution to the challenge facing the healthcare sector.

KEY WORDS: Fructosamine, diabetes mellitus, laboratory control, monitoring of treatment, diagnostics

INTRODUCTION

Technological progress enables the modification and enhancement of the various evaluation components of research monitoring. This has also been made possible by the development of chemical technologies that create many new reagents that affect the expansion of laboratory diagnostic capabilities [1]. However, even at this stage of development, there are diseases that, despite many studies, are still relevant in terms of the development of criteria that make it possible to compensate for them. Diabetes mellitus, due to its impact on the quality of life, and its possible serious complications, is a disease that requires improvement in terms of treatment approaches to long-term stabilisation of patients' state and successful laboratory diagnosis. After all, through assessment monitoring at the laboratory level, it is possible to timely detect tendencies indicating an improving, stabilising or, on the contrary, a negative state, which requires adjustments in therapeutic methods or the use of new diabetes treatment technologies that are currently being developed and implemented in the clinic [2]. Thus, the testing of their efficacy in the therapeutic and prophylactic effect on the patient, on which compensation of the identified indicative clinical and metabolic borderline is based, depends directly on the qualitative characteristics in providing all parameters reliability in obtaining values of certain diagnostic parameters characteristic of diabetes mellitus.

Currently, there are different diagnostic criteria for detecting the level of, for example, glucose, glycosylated haemoglobin (HbA1c), lipid profile or fructosamine [3]. Common tests for glucose level determination occasionally present errors of either its underestimation or overdiagnosis, which results in the administration of incorrect doses of medication, which can aggravate the condition of the patient. However, the normative limits of variation in glucose concentration differ significantly depending on the type of blood sample tested, as they may contain different amounts of glucose substance concentration, and this becomes a cause of erroneous evaluation criteria in the diagnosis of diabetes mellitus amidst interpretative error, essentially leading to overdiagnosis of the disease. Glucose levels may also vary according to the emotional state of the patient, which tends to stabilise rapidly under favourable external influences.

Thus, the determination of glucose level does not allow to reflect the patient's condition in terms of biochemistry, that had been present a few days prior [4]. HbA1c study may be a part of the diagnosis and control of treatment in a long term, which is important in considering the effects of taking medications and their adjustments, based on exploring new technologies or a change of treatment. According to the data, glycosylated haemoglobin reflects the glucose values of up to 12 weeks. Therefore, this method can be a diagnostic criterion for compensation of carbohydrate metabolism in patients with diabetes mellitus. However, given the possibilities of laboratory diagnostics, in the values of glucose concentration, fructosamine will also be detected for up to 20 days, and its determination takes place a lot faster than HbA1c, with which haemoglobin must also be determined [5]. Thus, the features of determining the values of fructosamine are relevant for the evaluation component in monitoring the efficacy of diabetes mellitus treatment.

AIM

The purpose of the study is to create a model for monitoring the efficacy of diabetes mellitus treatment by determining the fructosamine levels.

MATERIALS AND METHODS

The diagnostic parameters of this scientific and medical investigation were determined by the methods of clinical and laboratory studies. At the initial stage, medical history taking, observations, and a standard clinical examination, including an ophthalmological examination were carried out. Further, all patients underwent laboratory testing for the determination of carbon metabolism parameters by ion-exchange chromatography, where glycated haemoglobin values were determined by latex agglutination and fructosamine values by automatic colorimetric method using nitrosonium tetrazolinone chloride according to standard investigation schemes [6]. The study of the identified indicators of these diagnostic criteria was carried out concomitantly in order to compile an evidence base, which would reflect the quantification of fructosamine in various conditions of patients undergoing treatment, either in compensation, treatment efficacy or inefficacy, given the accuracy of the indicators in detecting laboratory diagnosis, determining the various necessary criteria for the evaluation-diagnostic monitoring of HbA1c. All the data obtained in this study were documented in the individual patient records created for each patient.

The data were processed using the programme Statistica, version 10. The findings were presented in the form of the median (Me) and a mass fraction in percentage terms. The Kruskal-Wallis test was used to statistically evaluate the level of significance at $p<0.05$ of the independent groups. The results are also shown graphically in figures and tables. The study was conducted on the basis of the Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan. 68 patients with a history of diabetes of more than 1 year participated in this medical investigation. The age of the subjects, among which there were 42 females and 26 males, was from 18 to 34 years. The diagnosis of diabetes mellitus in all patients was made on the basis of general criteria, as fined by common guidelines established in 1999 by the World Health Organisation (WHO) expert committee on diabetes mellitus, which include a collection of history, clinical data and confirmatory biochemical tests. Prior to the study, all patients had signed written consent to participate in the trial, in accordance with the 2000 ethical standards of the Declaration of Helsinki. Exclusion criteria were severe clinical forms and complications of diabetes mellitus.

The investigation was carried out in three stages, the first stage being an analytical-theoretical study of methodological literature on aspects related to diagnostic criteria for diabetes mellitus, which could provide quality monitoring of compensation and efficacy of the treatment of the disease through the use of laboratory diagnostic methods, during which the actual problem, purpose, research methods and work plan were defined. In the second stage, clinical and laboratory studies were conducted on patients with diabetes mellitus, then the results were analysed, and conclusions were formulated. In the third stage, the obtained results and conclusions were clarified and systematised.

RESULTS

All criteria and parameters determined in this study were considered, and analysed in detail, both separately and as their resulting components. Together this starts the process of forming a model of fructosamine values for quality control of stabilisation and efficacy of diabetes mellitus treatment, which can be further applied in clinical practice, improving the quality of the healthcare system of Kazakhstan. The result of the study is understood to be the extent to which the model under consideration has been implemented in applied medicine. The model for determining fructosamine levels in long-term monitoring of drug therapy based on the clinical stabilisation of diabetic patients' condition was introduced into the area of medical supervision in several stages, covering aspects of monitoring of diabetes mellitus in laboratory tests in terms of the effectiveness of the treatment and the selection of medication, including insulin therapy. The data obtained were further statistically processed, and the next stage involved the development and testing of a model to determine the optimal values of fructosamine, followed by its subsequent implementation in practice, allowing to control the treatment and course of diabetes, thus, addressing an important issue in the health care sector in Kazakhstan. A total of 68 diabetic patients were included in the trial. The clinical examination showed that the duration of the disease in patients ranged from 3 to 10 years, and the patients were also found to have diabetic polyneuropathy (30.8%), diabetic polyneuropathy (13.2%) and rhinopathy (35.3%) (Table 1).

At the initial state, glycated haemoglobin was tested to allocate patients to groups based on the stage of carbon-metabolism compensation. The average glycated

Table 1. Clinical characteristics of patients

Criteria	Number of patients (n = 68)
Age, mean (min; max)	27 (10; 34)
Disease duration, mean (min; max)	6 (3; 10)
Diabetic polyneuropathy, abs (%)	21 (30.8%)
Diabetic retinopathy, abs (%)	24 (35.3%)
Diabetic nephropathy, abs (%)	9 (13.2%)

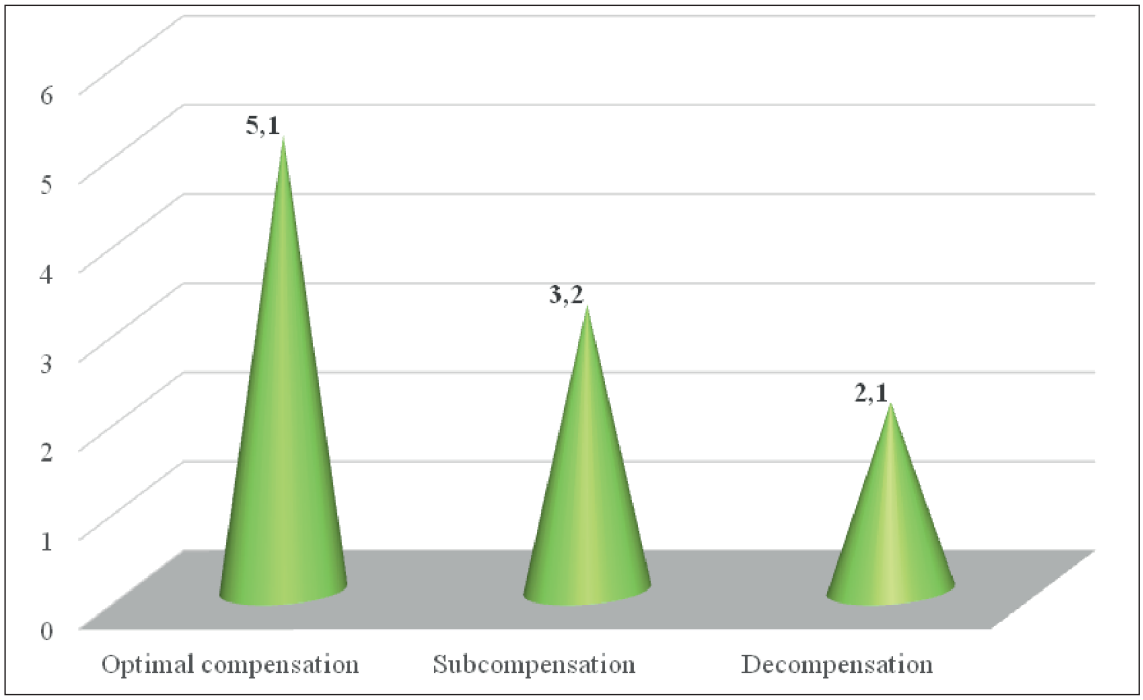

Figure 1. Distribution of patients based on quantitative self-monitoring of glycaemia

haemoglobin level was found to be 8.1 ± 0.18%. The HbA1c values were found to be comparable to the Gaussian curve, with a minimum of 6.1% and a maximum of 12.24%, where the median value was 8.67%. Patients were divided into groups based on metabolic compensation level, as shown in Table 2.

The examination identified a significant proportion of diabetes mellitus patients with decompensation. The self-monitoring glucose measurement frequency data were clarified, revealing that patients in the disease stage of compensation, subcompensation and decompensation determined blood glucose levels with varying regularity. The results are illustrated in Figure 1.

The data were then used to adjust the complex treatment therapy, including the correction of physical activity routine, diet, and drug therapy, which was initially supported by an additional daily self-monitoring of the glycaemic level. The gradual correction of therapeutic measures for stabilising the condition of diabetic patients was overseen by clinical and laboratory tests, determining glycated haemoglobin and fructosamine levels. The observation phase lasted for three months from the start of the research. The methodological analysis of the data obtained suggests that medical institutions should periodically monitor diabetic patients to determine their carbohydrate metabolism status. The study has also shown, that over time patients may get used to their pathological condition and conduct periodic self-monitoring mechanically, unaware of the dangerous consequences of changing glucose concentrations and patterns, sometimes even as small as compromising a strict approach in choosing a certain group of foods or in reducing their physical exercise regime. Given the essentially lifelong use of medication and insulin, diabetic patients become accustomed to this factor becoming part of their lives, and over time, as research data shows, they tend to forget to adjust daily dosages of insulin and other medications, which is mandatory for this patient population. After all, further therapeutic factors will depend on the detected level of glycemia, which will contribute to prolonging the stage of compensation for diabetes mellitus diseases and will maintain a relatively healthy level of the patient's vital systems, which are essentially its target in decompensation [7].

Thus, timely monitoring and adjustment of optimal doses of drugs and other means of treatment efficacy will ensure that patients do not develop severe complications of diabetes, prevent the development of disability, and prolong and maintain a higher quality of life for these patients. Thus, based on the assumption that at some

point in their illness patients start to diminish the level of personal control over their condition, this parameter should be introduced into the sphere of medical control, where specialists will determine the level of efficacy and relevance of the treatment provided based on the stabilisation of the patient's condition. Such check-ups will also allow the doctor to remind patients of the importance of self-monitoring of blood glucose levels and maintaining a diet, active physical routine, and other aspects of a healthy lifestyle. This will also help to familiarise patients with new methods of treatment, which, if necessary, can be applied depending on their condition, regulating, and maintaining stable carbohydrate metabolism [8]. It is important to have a laboratory indicator that allows the physician to judge on the efficacy of the treatment administered by the patient, in which case fructosamine values will allow the physician to undertake this monitoring every three weeks, with subsequent targeted adjustments to treatment or regimen conditions.

Thus, the facts described above dictate the need to develop a model for the application of fructosamine indicative values that would reflect the necessary factors for medical control in diabetic patients. The realisation of the study requires methodical analysis and allows to determine its main parameters, which based on a generalised approach of maintenance of treatment efficiency and formation of optimal metabolic compensation, forms a data system of fructosamine value determination within the framework of periodic control in two to three weeks, which will improve the overall quality of the existing healthcare programme in Kazakhstan in terms of the quality of health care provision for this patient population.

Given the above, a model for the use of fructosamine parameters was developed to monitor the efficacy of treatment and patients' condition, for the timely correction of various criteria affecting the compensation, where there is a direct correlation between patient well-being, development of complications, disabilities, and quality of life. In this case, the periodic and timely determination of fructosamine values will allow the professional healthcare service to retrospectively measure the impact of the patient's treatment in terms of its quality and efficacy and maintain a stable state of carbohydrate metabolism. If necessary, this will be adjusted by the physician on a regular and professional basis with the application of treatment agents based on new research data or treatment methods, where these parameters will also be regularly, once every 3 weeks, monitored against the identification of treatment effect on the patient in the fructosamine value determination criteria. A developed model for adjustments in therapy is based on a retrospective determination of the efficacy of the ongoing treatment in the sample of fructosamine values. This method could be included in the official list of recommendations of the Consensus on the Diagnosis and Treatment of Diabetes Mellitus in Kazakhstan and improve the productivity of this area of health care.

Then, in a control phase of the study, the developed model for determining fructosamine parameters was tested for three months, and so every three weeks its quantitative parameters were determined. Glycated haemoglobin tests contributed to the collection of data in the criteria for comparison and reliability of the obtained fructosamine values, on the background of conducted treatment determining the level of compensation of carbohydrate metabolism, as well as the implementation of the necessary adjustments in medications, which made it possible to gradually improve patient's condition. Given that the study period was three months, laboratory fructosamine tests and clinical examinations were carried out 4 times. At each follow-up visit, based on the findings, additional adjustments were introduced into the treatment of diabetes mellitus every three weeks, with a focus on the normalisation of carbohydrate metabolism and the correction of the associated factors based on the patients' lifestyle. Further, the study presents systematised data of 4 control visits with the distribution of patients according to the level of stabilisation of their condition, within the selected type of treatment in assessing the level of its efficacy. Thus, table 2 presents fructosamine and HbA1c levels at the first follow-up, revealing relevant data on the determination of metabolic compensation and fructosamine parameters.

Table 3 presents the data from the second follow-up visit, where the findings reveal a positive trend in stabilisation. It should also be noted that the quantitative indicators of patients presented in the Table 3 varied depending on the fructosamine level, however, the level of HbA1c remained the same.

Table 4 shows the results of day 3, where a significant improvement in the condition of diabetic patients was detected, confirming the correct strategy based on the comparison of laboratory data and the adjustments to drug therapy.

Table 5 shows level 4 medical monitoring indicators in the efficacy of the developed model, highlighting the importance of monitoring the administration of medicines and treatment in general by medical staff, based on the laboratory and clinical parameters of the patient.

It should be noted that based on the HbA1c values during ongoing treatment, a visible change in the metabolic compensation would not be fully reflected due to the time factor, making the fructosamine values indicative in this context. Also, the analysis of the presented data throughout the study revealed a positive trend in the efficacy of treatment adjustments, which created conditions for stabilising the condition of diabetes patients. Thus, based on the findings shown in Table 6, all patients were advised to continue periodic follow-ups with medical professionals and regular fructosamine testing, which helps to determine the correct treatment strategy based on its complex efficacy, resulting from the administration of medication and insulin, daily glucose level self-checks, diet and exercise, positive psycho-emotional environment by avoiding negative influences on one's emotional state and general well-being [9]. Also, at the final stage of the study, patients' attitudes towards periodic monitoring and adjustments to their treatment strategy turned positive, as shown in Figure 2.

Table 2. Distribution of patients by the level of carbohydrate metabolism compensation

Parameter	Compensation level		
	Optimal compensation 1 g	Subcompensation 2 g	Decompensation 3 g
Number of patients (n = 68), Abs (%)	19 (27.9%)	32 (47%)	17 (25.1%)
HbA1c (level)	6.10%	7.10%	11.20%
Fructosamine (mmol/l), mean±SD	2.6 ± 0.9	3.2 ± 0.3	4.8 ± 0.7

Table 3. Distribution of patients by the level of carbohydrate metabolism compensation at the 2nd control level

Parameter	Compensation level		
	Optimal compensation 1 g	Subcompensation 2 g	Decompensation 3 g
Number of patients (n = 68), Abs (%)	21 (30.9%)	41 (60.3%)	6 (8.8%)
HbA1c (level)	6.10%	7.10%	11.20%
Fructosamine (mmol/l), mean±SD	2.5 ± 0.7	3.2 ± 0.6	4.9 ± 0.4

Table 4. Distribution of patients by the level of carbohydrate metabolism compensation at the 3rd control level

Parameter	Compensation level		
	Optimal compensation 1 g	Subcompensation 2 g	Decompensation 3 g
Number of patients (n = 68), Abs (%)	35 (51.5%)	29 (42.6%)	4 (5.9%)
HbA1c (level)	5.10%	7.10%	10.10%
Fructosamine (mmol/l), mean±SD	2.5 ± 0.4	3.1 ± 0.9	4.6 ± 0.7

Table 5. Distribution of patients by the level of carbohydrate metabolism compensation at the 4th control level

Parameter	Compensation level		
	Optimal compensation 1 g	Subcompensation 2 g	Decompensation 3 g
Number of patients (n = 68), Abs (%)	55 (80.9%)	12 (17.6%)	1 (1.5%)
HbA1c (level)	5.10%	7.10%	10.10%
Fructosamine (mmol/l), mean±SD	2.5 ± 0.3	2.9 ± 0.7	3.9 ± 0.6

Thus, the analysis of the obtained data showed the efficacy of the developed model, as the fructosamine factor allows for timely correction of treatment, as part of the monitoring at three weeks diagnostic intervals. The present study validity was ensured as the characteristics and parameters of the clinical and laboratory studies, the data obtained, and the model developed were correctly compared in the investigation, and the analysis of its results determined that the study results can contribute to the sphere of clinical practice within the framework of improving diabetes mellitus rates. Thus, summarising the data obtained, fructosamine levels normally range between 2 and 2.8 mmol/l, for people with controlled diabetes, the fructosamine range should be from 2.8 to 3.7 mmol/l, and with values of higher than 3.7 mmol/l at the decompensation level [10]. When treatment is ineffective, fructosamine levels will increase, and with a carefully selected and adjusted diet and lifestyle, fructosamine values will decrease to normal levels. This makes a determination of fructosamine values an important marker in determining glycaemia, with its relevance and efficacy having been identified and confirmed in a long-term study.

The fructosamine testing can thus be used for qualitative monitoring of diabetes mellitus in order to determine the efficacy of its treatment and, if necessary, its correction until the patients' condition is stabilised. Also, fructosamine level tests are essential when changing routine treatment, during pregnancy in diabetic patients, in anaemia and after blood loss. Follow-ups by healthcare professionals are also important to detect errors in treatment practices by patients, ruling out or reducing the development of complications from diabetes mellitus. Thus, the model developed allows the monitoring of diabetes mellitus treatment, determining the level of its efficacy, and the formation of sustained compensation of the disease with the implementation of a competent selection of therapy and its effective components, which is of practical importance for health care.

DISCUSSION

Based on the system-activity approach of this study, a detailed analysis of the clinical and laboratory data was obtained, which allows reflecting the factors of a certain manifestation of diabetes mellitus excluding complications

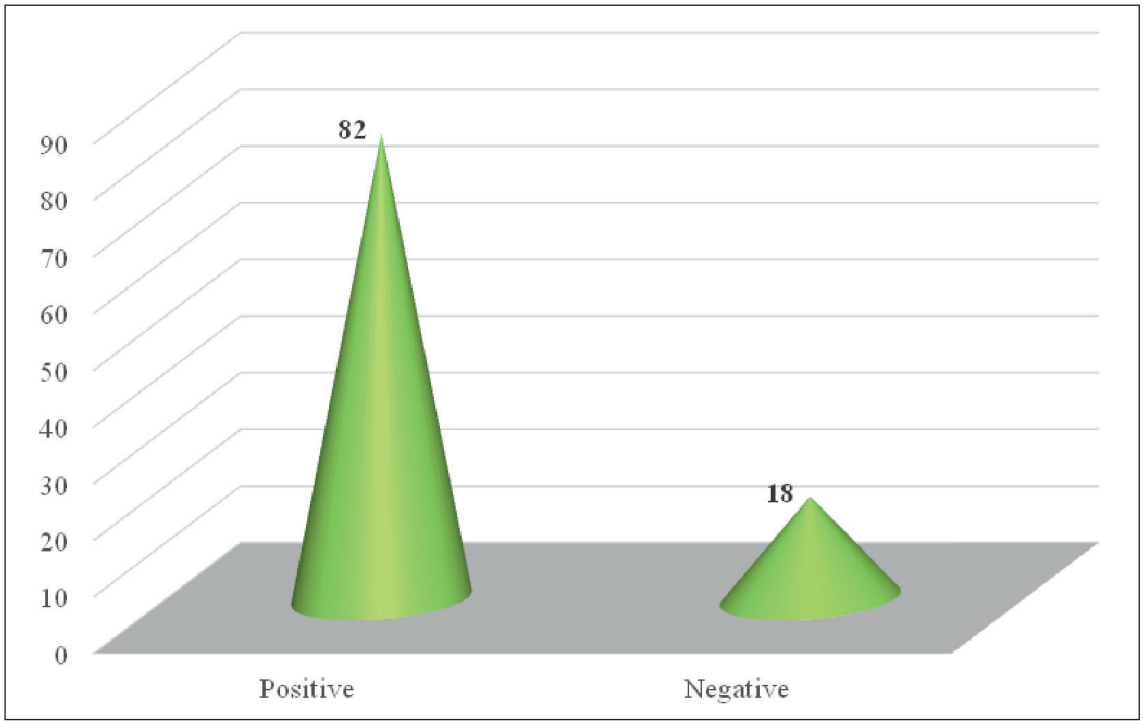

Figure 2. Distribution of patients according to their attitudes towards follow-ups and adjustments to their treatment

which may trigger a serious exacerbation, and so the presence of the main manifestations of the disease was found in them, which was confirmed by laboratory tests in the criteria for the determination of valid indicators within the framework of the detection of glycated haemoglobin values, and a concomitant analysis of fructosamine values. Glycated haemoglobin is the official diagnostic criterion for the diagnosis of the disease, as recommended by the WHO in 2002, so the ratio of its isolated values against a background of diabetes mellitus monitoring reflects the patient's condition in a retrospective analysis of the carbon metabolism over several months [11]. In addition to the established diagnosis, which determines the factors for the onset and presence of the disease, there are important aspects determining the efficacy of treatment, resulting in metabolic compensation, which translates into improved quality of life of patients and helps to prevent the serious complications common to diabetes mellitus, the development of disabilities and premature mortality. Thus, evaluative monitoring of the course of the disease is an important part of the ongoing treatment, and in considering a serious disease such as diabetes mellitus, it will enable the selection of optimal treatment that will form the compensation of the disease. This is also important in view of the development of the new methods of treatment in order to identify their effect on the course of the disease in each individual case [12].

The determination of glycated haemoglobin levels provides a retrospective view of the patient's condition over the last few months, which reflects the progression of the disease and markers such as medication therapy, exercise, diet, and other components that qualitatively influence the clinical course of diabetes, as HbA1c reflects the glucose concentration values over the life of the erythrocyte. Other conditions that may affect the glycated haemoglobin values include bleeding, anaemia, or chronic kidney disease, so patients with these conditions also require other tests to monitor their condition [13]. Along with alike qualitative characteristics of glycated haemoglobin, and similar as a diagnostic criterion, not changing in their indicative values based on the described pathological conditions, but equally allowing for display in the factor of monitoring treatment efficacy and stabilising the condition, is the detection of fructosamine values [14]. It is currently considered an alternative method of investigation, given the degree of informative, revealing characteristics of this criterion, which also determines the glycaemia level that the patient has had for the past few weeks. As here fructosamine is an indicative diagnostic element, it is important in determining the efficacy of treatment and in terms of identifying the values that will be characteristic of stabilising the course of diabetes and for the selection of drug therapy [15].

Fructosamine is a serum glycated blood protein, and it is a qualitative indicator of blood glucose levels. Its formation in the blood serum is associated with an irreversible non-enzymatic process, where blood proteins bind, to a greater extent from the albumin protein with glucose [16]. This reaction occurs independently of insulin. However, there is a difference in the time of determination based on the fact that the half-life of the detected serum protein values is shorter than that of the whole red blood cell life cycle, on which the glycated haemoglobin levels depend, and which can also be determined in a time interval of up to three months. Thus, serum fructosamine values may reflect the glucose concentration at its average levels for as long as the protein circulates in the blood. This period lasts from one to three weeks, and the indicator depends

on the type of protein with which the glucose interacts, as the half-lives of their type differ from one another. Thus, the fructosamine values indicate the level of short-term or permanent elevation of glucose, on which the level of glycosylation of serum proteins also depends at the biochemical level. It is recognised that the greater the mean glucose level is in a retrospective view of two-three weeks, the greater the degree of fructosamine concentration will be, and this also has an inverse relationship [17].

Thus, with the aggravation of diabetes mellitus symptoms, there will also be an increase in the concentration of fructosamine, and so the degree of its decrease will reflect stabilisation from the standpoint of improving the clinical course of this disease. It should also be noted that fructosamine values do not depend on a high level of glucose concentration in the blood, which can be triggered, for example, by stress or a highly excited state, which is significant in diagnostic criteria for the efficacy of treatment or stabilisation of patients, as these are detected without interference from various psycho-emotional disturbances to which glucose is in turn sensitive. This makes determining fructosamine values a qualitative and highly reliable component of condition monitoring in diabetes mellitus [18]. Thus, even in the case of insulin therapy, this study would allow stable glucose levels to be detected, which is of practical importance in determining the optimal doses or type of insulin, which at the clinical level will contribute to maintaining glucose concentrations close to normal values, making it the key to stability and efficacy of diabetes treatment, which will prolong the stage of clinical remission of the disease in patients and prevent many serious complications that have a qualitative impact on their quality of life.

Another benefit of testing fructosamine levels is that patients are not required to fast before the test, as its values reflect glucose levels in previous weeks, and food eaten on the day of testing will not be reflected in the test results [19]. In spite of the many advantages of fructosamine testing described above, compared to HbA1c, which is also more difficult and rare in laboratory diagnosis due to updating in data and additional steps in haemoglobin testing, even considering the great achievements in modern laboratory diagnostics, the determination of fructosamine values is not common in clinical practice. This is largely due to the lack of criteria for long-term monitoring based on its determination in the factor of drug intake and treatment in general [20-22].

CONCLUSIONS

In today's world, given technology advancements for the treatment of diabetes mellitus, there is a need to develop a method that allows the timely detection of the efficacy of treatment, and at the same time, as part of the evaluative monitoring of medical control, determines the effectiveness of the drugs prescribed with the aim of formation of stable metabolic compensation. The model developed in this study allows determining fructosamine values in diabetic patients to monitor the efficacy of treatment, where doctor conducting regular check-ups can determine the criteria for stabilising the condition of the patient based on various factors such as diet, physical activity, regular and correct intake of medicines. Timely adjustments to treatment will allow for the stabile compensation, thus maintaining a high quality of life and reducing the development of various complications and disabilities. In this way, the patient's fructosamine levels will be measured at regular visits, once every three weeks, and thus the effectiveness of the treatment provided will be assessed. The developed model, when implemented in the health sector, will improve medical care for patients with diabetes mellitus. The materials of this study will be useful for healthcare professionals and can be applied in practice, thus contributing to solving important challenges in medical care for diabetic patients.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

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EXPERIMENTAL AND MORPHOLOGICAL ASSESSMENT OF THE INFLUENCE OF HYDROXYAPATITE-CONTAINING OSTEOTROPIC MATERIAL AND ELECTRICAL STIMULATION ON REPARATIVE OSTEOGENESIS OF THE LOWER JAW

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ABSTRACT

Aim: The aim of the study was to reveal the peculiarities of reparative osteogenesis in experimental lower jaw defect under the conditions of hydroxyapatite-containing osteotropic material application and electrical stimulation.

Materials and Methods: An experiment was conducted on 48 mature male rats of the WAG population. All animals were divided into 4 groups (12 animals in each group). Group 1 included rats that were not subjected to any manipulations. Group 2 included rats that were modeled with a perforated defect of the lower jaw body. Group 3 included rats that were modeled with a perforated defect similar to group 2, the cavity of which was filled with synthetic bone graft "Biomin GT" (RAPID, Ukraine). Group 4 included animals that were modeled with a perforated defect similar to groups 2-3, the cavity of which was filled with synthetic bone graft "Biomin GT". In animals of group 4, a microdevice for electrical action was implanted subcutaneously in the neck area on the side of the simulated bone defect. Morphological and statistical methods were used.

Results: The research carried out by the authors proved that the use of the above-mentioned bone replacement material helps to increase the regenerative potential of the bone tissue of the lower jaw, but does not lead to the formation of a full-fledged bone regenerate, as evidenced by the results of the morphometry of the regenerate (the specific volume of lamellar bone tissue accounted for 54.9%); disordered localization of bone beams, which were characterized by reduced signs of mineralization; the presence in connective, osteogenic fibroreticular and lamellar bone tissues the encapsulated bone graft granules with the presence of inflammatory cell infiltration. In cases the combined use of synthetic bone graft "Biomin GT" and electrical stimulation, the authors noted more intensive reparative osteogenesis processes in the bone defect of the lower jaw compared to cases when only one bone graft was used, but they also did not lead to the formation of a full-fledged bone regenerate.

Conclusions: The experimental and morphological study conducted by the authors proved that the use of hydroxyapatite-containing osteotropic material ("Biomin GT"), especially in cases of its combined use with electrical stimulation, significantly activates reparative osteogenesis in the bone defect of the lower jaw, which does not lead to the formation of a full-fledged bone regenerate.

KEY WORDS: hydroxyapatite-containing osteotropic material, electrical stimulation, experiment, morphology, reparative osteogenesis, lower jaw

INTRODUCTION

Mandible bone defects still poses a major problem in the field of oral and maxillofacial surgery that may be caused by trauma, inflammatory processes, congenital malformations, tumors, cyst formation and other pathological factors [1, 2]. Mandibular defects can lead to facial deformities, severe maxillofacial dysfunctions, decrease in the quality of patients life [3, 4].

The bone has a great reparative potential, however, in cases of large defects, self-healing does not occur, which dictates the need to use various synthetic osteotropic materials to replace the bone defects and stimulate the reparative osteogenesis [5]. Modern osteotropic materials should be characterized by the following properties: biocompatibility; ability to resorption and replacement by bone tissue, to rapid integration with it, to osteoconductivity and osteoinductivity; relative ease of introduction into the defect site and the possibility of modeling during surgery [6]. The use of various osteotropic materials in clinical practice is a difficult task due to their incompletely studied efficacy, which can lead to suboptimal regeneration, long healing time, low quality of the newly formed bone, etc. [7]. Numerous studies demonstrate the high effectiveness of the use of complex osteotropic materials based on hydroxyapatite for replacing bone defects in dentistry, traumatology and veterinary medicine [8, 9]. Today, the quality of the formed bone regenerates filling the defects in the

lower jaw when using the hydroxyapatite-containing materials is not fully studied.

The search of reparative osteogenesis stimulating methods is an actual problem of oral and maxillofacial surgery, orthopedics and traumatology. The use of biological factors, in particular electric current, is a promising alternative method for stimulating the reparative osteogenesis in patients with mandibular defects, its effectiveness and action mechanisms are debatable and not fully understood [10].

AIM

The purpose of the study was to reveal the peculiarities of reparative osteogenesis in experimental lower jaw defect under the conditions of hydroxyapatite-containing osteotropic material application and electrical stimulation.

MATERIALS AND METHODS

An experiment was conducted on 48 mature male rats of the WAG population. All animals were divided into 4 groups (12 animals in each group).

Group 1 included rats that were not subjected to any manipulations.

Group 2 included rats that were modeled with a perforated defect of the lower jaw body. Anesthetized rats underwent a 1.0-1.2 cm long incision of the skin, subcutaneous tissue, and superficial fascia in the left submandibular area. A fragment of the outer surface of the branch and body of the lower jaw was skeletonized. With a ball-shaped drill and a straight tip with a diameter of 3.0 mm, a transcortical perforated defect of the body of the lower jaw was formed in the form of a channel, departing from the lower edge of the jaw upwards by 2 mm. The wound was sutured in layers with vicryl.

Group 3 included rats that were modeled with a perforated defect similar to group 2, the cavity of which was filled with synthetic bone graft "Biomin GT" (RAPID, Ukraine), which includes hydroxyapatite and β-tricalcium phosphate. After filling the bone defect with a bone graft, the wound was sutured in layers with vicryl.

Group 4 included animals that were modeled with a perforated defect similar to groups 2-3, the cavity of which was filled with synthetic bone graft "Biomin GT", and the wound was sutured in layers with vicryl. In animals, a microdevice for electrical action was implanted subcutaneously in the neck area on the side of the simulated bone defect (a temporary Videx AG 4 battery; a constant sinusoidal electric current of an unchanging nature 1 milliampere, frequency 30 W). The negative electrode connected to the negative pole of the battery was in contact with the bone defect. The battery and electrode were insulated with plastic heat shrink material.

Animals of groups 1-4 were removed from the experiment on 3, 7, 14 and 28 days (3 animals for each experimental period).

The material for the morphological study was a fragment of the body of the intact lower jaw in group 1, and in groups 2-4 – from the zone of the perforated defect modeling. The material was fixed in a 10% solution of neutral formalin (pH 7.4) for 24-48 hours, decalcified, carried out according to the generally accepted method and embedded in paraffin. From paraffin blocks, serial sections with a thickness of 4-5 μm were made, which were stained with hematoxylin and eosin, picrofuchsin according to van Gieson.

Examination of the microslides was carried out using a laboratory microscope ZEISS Primostar 3 (Carl Zeiss, Germany) with a built-in color digital camera. Morphometry was carried out using the Labscope program, during which the specific volumes of the structural elements of the regenerate were calculated at different experimental periods.

The indicators in the groups were processed statistically using the Statistica 10.0 program. Mean values of indicators in groups were compared using the non-parametric Mann-Whitney U-test. Differences were considered significant at $p<0.05$.

RESULTS

On the 3rd day of the experiment, survey microscopy revealed a bone defect extending through the entire thickness of the lower jaw in rats of groups 2-4. In the simulated bone defect, fragments of blood clots, altered particles of the epithelial layer, muscle, connective and bone tissues were noted, with the presence of bone graft granules among them in groups 3 and 4 (Figs. 1, 2). Alteratively changed tissues were diffusely infiltrated by neutrophilic leukocytes, macrophages, lymphocytes, histiocytes and sometimes cells of the fibroblastic series. Small foci of immature granulation tissue were noted at the edges of the bone defect in all groups, as well as around the graft granules in groups 3 and 4. The granulation tissue was characterized by a pronounced cellular component, a moderately pronounced vascular component, and an unexpressed fibrous component. The cellular component of the latter was represented by neutrophilic leukocytes, macrophages, lymphocytes, histiocytes and fibroblastic differon cells; vascular – vessels of different shapes and diameters; fibrous – thin bundles of connective tissue fibers. In group 2, the granulation tissue was characterized by pronounced hemodynamic disturbances, represented by vascular hyperemia, edema and hemorrhages.

During the morphometric study, it was determined that on the 3rd day of the experiment, the predominant elements in the bone cavity in group 2 were a blood clot and altered tissues, in groups 3 and 4 – granulation tissue and altered tissues (Fig. 3). During the comparative analysis, it was established that the specific volume of the blood clot was greater ($p<0.05$) in group 2 compared to groups 3 and 4; the specific volume of altered tissues was smaller ($p<0.05$) in group 4 compared to group 2; the specific volume of granulation tissue was greater ($p<0.05$) in group 4 compared to group 3, in group 3 compared to group 2. The parameters obtained by the authors indicated that in group 3, and especially in group 4, compared to group 2, in the bone cavity took place more intensive processes of cleaning it from the blood fragments and altered tissues, as well as filling it with granulation tissue.

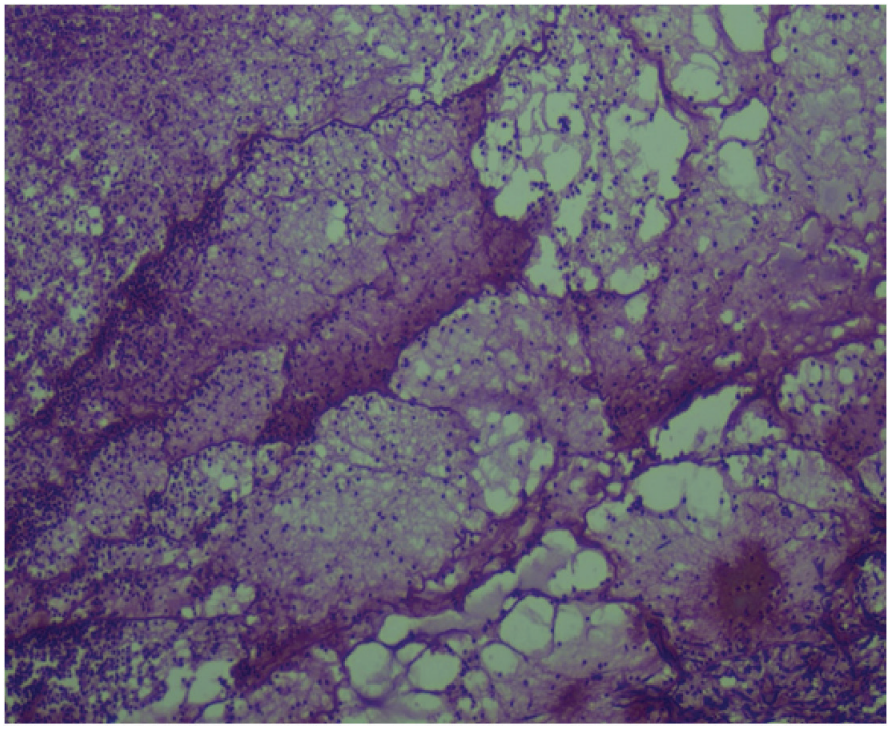

Fig. 1. Group 2. Blood clot fragments and foci of immature granulation tissue in the bone defect cavity. Hematoxylin and eosin staining, ×100.

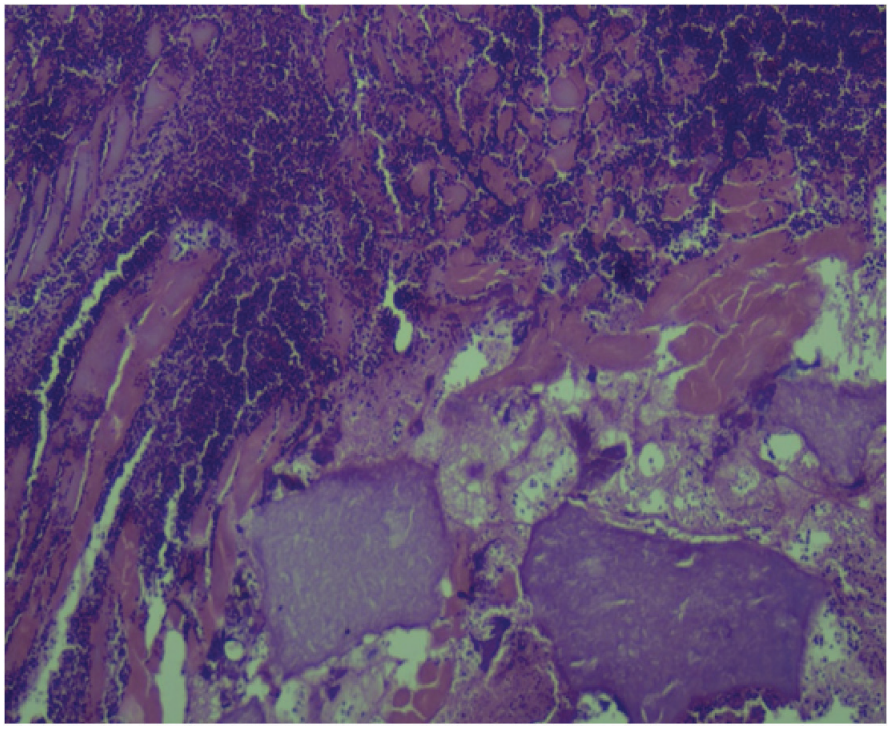

Fig. 2. Group 4. Alteratively changed connective and muscle tissue with diffuse polymorphic cellular infiltration, bone graft granules in the bone defect cavity. Hematoxylin and eosin staining, ×100.

On the 7th day of the experiment, survey microscopy revealed in groups 2-4 the remains of a blood clot in the bone cavity, which was mainly represented by fibrin threads and hemolyzed erythrocytes; altered fragments of connective, muscle and bone tissues that were diffusely infiltrated by neutrophilic leukocytes, macrophages, lymphocytes, histiocytes and fibroblastic differon cells. Granulation tissue of various degrees of maturity and connective tissue were also determined in the formed cavity. Proliferation of osteoblastic elements and the formation of osteogenic fibroreticular tissue (Fig. 4), which filled the cavity, were recorded on the perio- and endosteum surface of intact bone

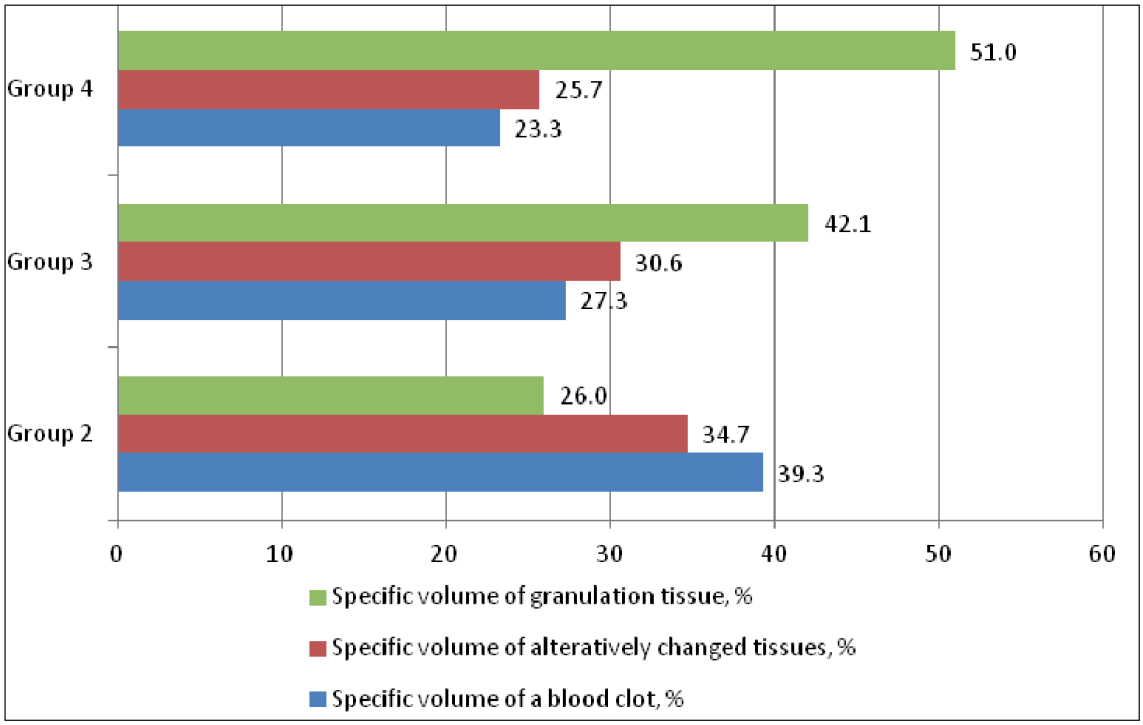

Fig. 3. The average values of specific volumes of elements (%) filling the bone cavity in the lower jaw of animals of groups 2-4 on the 3rd day of the experiment.

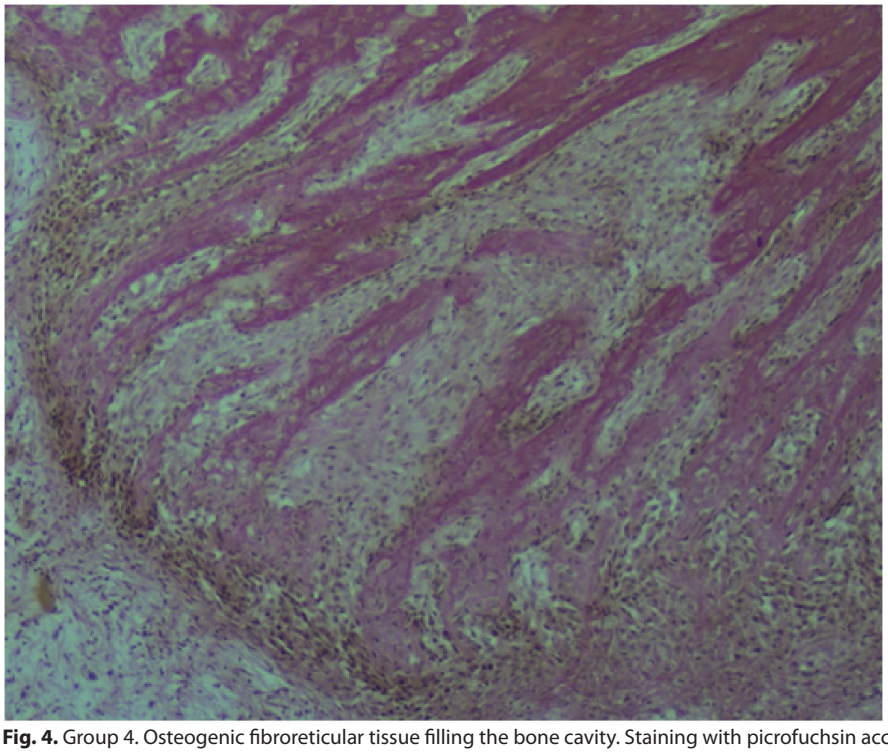

Fig. 4. Group 4. Osteogenic fibroreticular tissue filling the bone cavity. Staining with picrofuchsin according to van Gieson, ×400.

tissue bordering the cavity. In the foci of granulation and connective tissues in groups 3 and 4, bone graft granules were found (Fig. 5), around which clusters of osteoblasts were often identified together with the formation of osteogenic fibroreticular tissue. There were no signs of biodegradation of bone graft granules. A pronounced polymorphic cellular infiltration was often found around the bone graft, which was represented by neutrophilic leukocytes, macrophages, lymphocytes, histiocytes, fibroblastic differon cells, and foreign body-type multinucleated giant cells.

The results of the morphometric study of the specific volumes of the elements that filled the bone cavity of the lower jaw of animals of groups 2-4 on the 7th day of the experiment are shown in Figure 6. The cavity was mostly

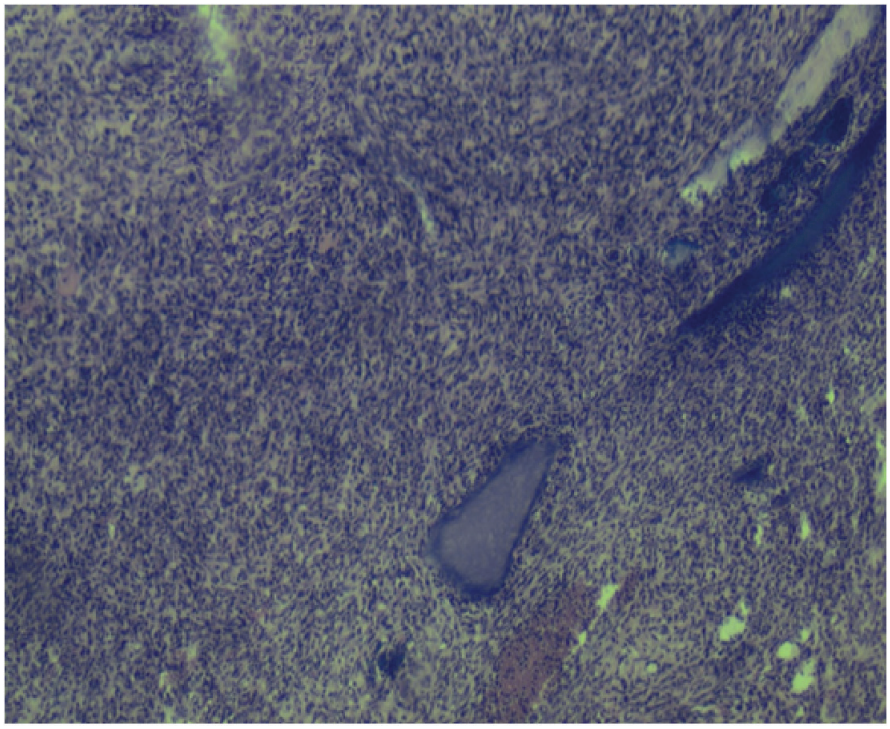

Fig. 5. Group 3. Bone graft granules in the foci of granulation tissue. Hematoxylin and eosin staining, ×100.

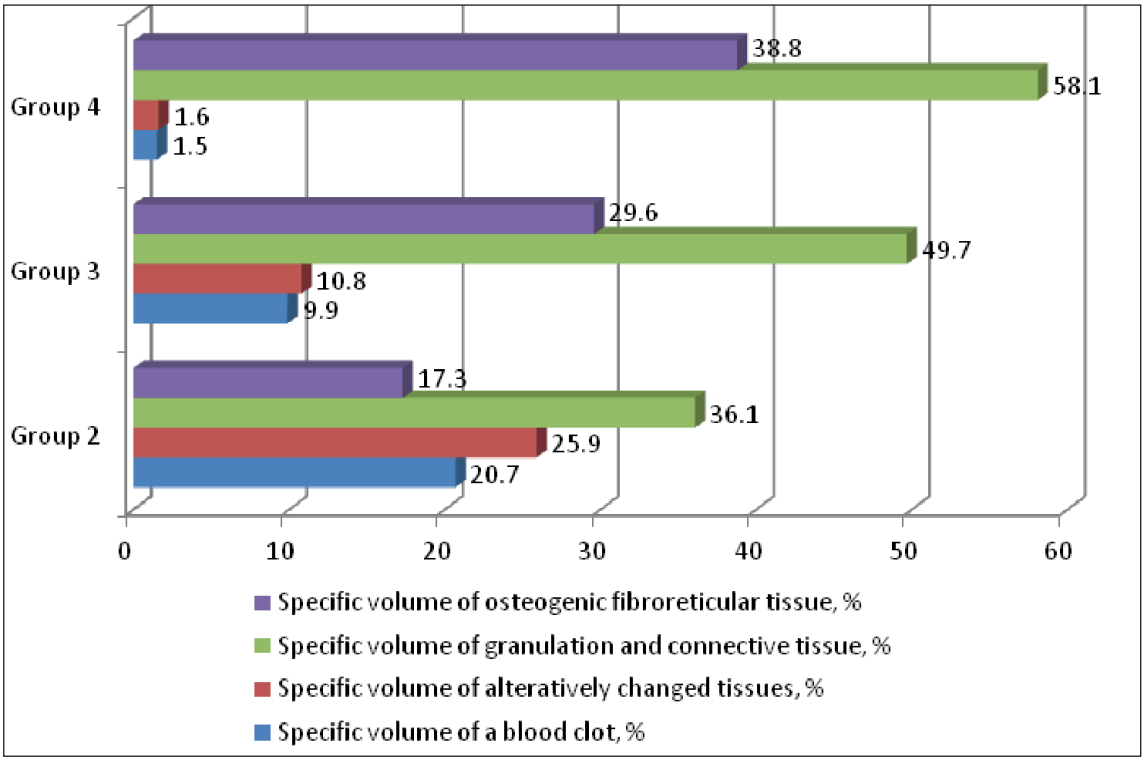

Fig. 6. The average values of specific volumes of elements (%) filling the bone cavity in the lower jaw of animals of groups 2-4 on the 7th day of the experiment.

filled, according to Figure 6, in group 2 with granulation and connective tissues, altered tissues; in groups 3 and 4 with granulation and connective tissues, osteogenic fibroreticular tissue. The conducted intergroup comparative analysis of the obtained indicators during the given period of the experiment testified more active processes of cleaning and healing of the bone defect of the lower jaw in group 3 and especially in group 4 compared to group 2.

On the 14th day of the experiment in groups 2-4, no remnants of a blood clot or altered tissues were found

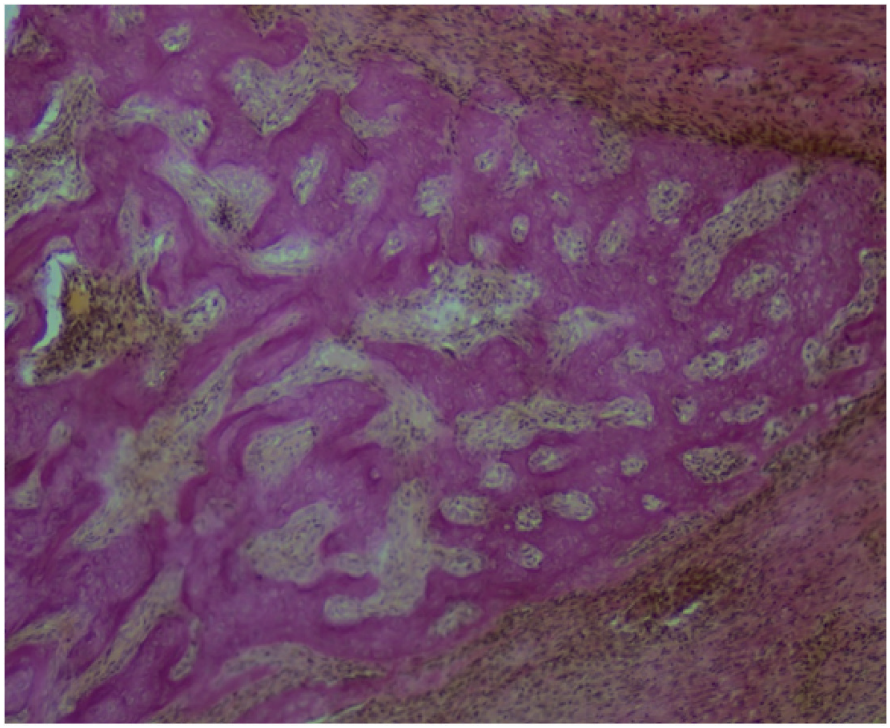

Fig. 7. Group 3. Lamellar bone tissue in the regenerate. Staining with picrofuchsin according to van Gieson, ×100.

in the bone cavity. In group 2, the bone regenerate was represented by granulation tissue of various degrees of maturity, connective tissue and osteogenic fibroreticular tissue. In group 3, the regenerate filling the bone cavity was represented by mature granulation, connective and osteogenic fibroreticular tissues. The latter in some visual fields turned into the lamellar bone tissue (Fig. 7).

In group 4, the regenerate was represented by connective tissue, lamellar and fibroreticular bone tissue. In lamellar bone tissue, the intertrabecular spaces were filled with connective tissue, with the presence of hematopoietic foci in some of them. In group 4, the areas of metaplasia of connective tissue into bone tissue were found. In groups 3 and 4, bone graft granules were visualized, the number of which did not change compared to the previous term. Active processes of osteogenesis were noted around some of the bone graft granules. However, a connective tissue capsule was formed around some of the granules with the presence of neutrophilic leukocytes, lymphocytes, histiocytes, and foreign body-type multinucleated giant cells (Fig. 8).

During morphometry, it was determined that granulation, connective and lamellar bone tissues prevailed in the regenerate in group 2; in groups 3 and 4 – lamellar bone and osteogenic fibroreticular tissues (Fig. 9). The comparative intergroup analysis of the obtained indicators proved that the bone defect healing processes took place more actively in group 3 and especially in group 4 compared to group 2.

On the 28th day of the experiment, the regenerate in groups 2-4 was represented by connective, osteogenic fibroreticular and lamellar bone tissues. In the specified tissues in groups 3 and 4, bone graft granules were visualized, the number of which did not change compared to the previous periods of the experiment. A connective tissue capsule with lymphohistiocytic infiltration was formed around the granules in some visual fields. In group 2, and sometimes in groups 3 and 4, bone beams in the lamellar bone tissue of the regenerate did not have an orderly spatial orientation, were colored unevenly, not as intensely as in group 1, which indicated a decrease in mineralization and density of this tissue. Between the bone beams, connective tissue was localized in some places with the signs of hematopoietic cell population, which was more pronounced in group 4 compared to groups 2 and 3.

Figure 10 shows the results of the morphometric study of the regenerate. The latter in all groups was characterized by the predominance of lamellar and fibroreticular bone tissues. Reparative osteogenesis, as in the previous terms, occurred more intensively with the formation of higher-quality regenerate in group 3, and especially in group 4 compared to group 2.

Comparative analysis of the obtained indicators in groups 2-4 in dynamics during the studied period (from 3 to 28 days) revealed their changes due to the healing processes of the bone defect.

DISCUSSION

The research carried out by the authors showed that the reparative osteogenesis in lower jaw is a complex and staged process, which also coincides with the point of view of other scientists [11]. The key chains of reparative osteogenesis are resorption of damaged tissues due to inflammation, cells differentiation, their proliferation and the formation of a new bone with its subsequent remodeling under the influence of biomechanical factors, the formation of an organic extracellular matrix and its mineralization [12].

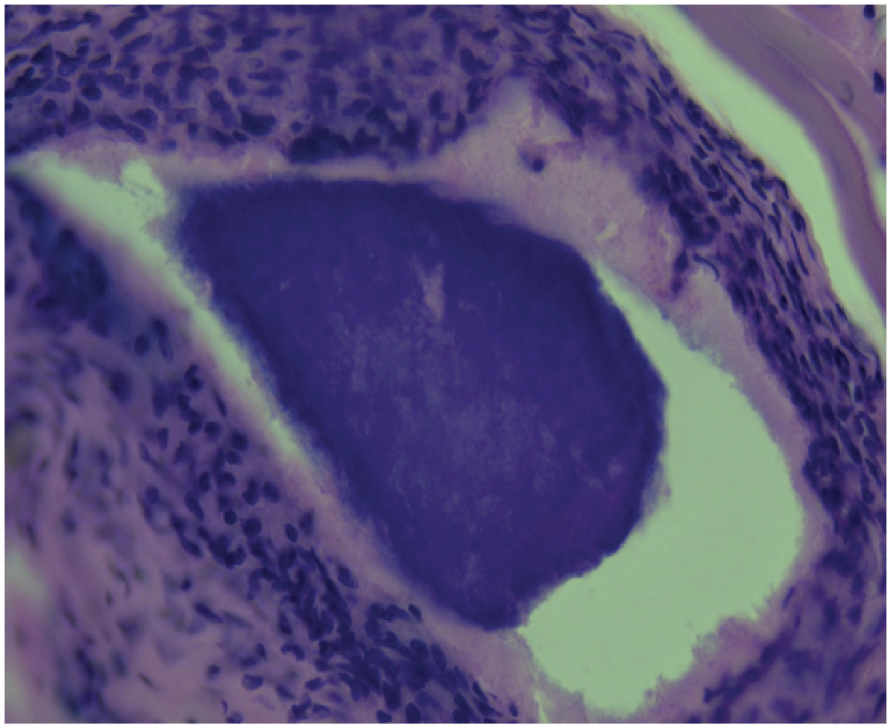

Fig. 8. Group 3. Connective tissue capsule with polymorphic cellular infiltration around the bone graft granule. Hematoxylin and eosin staining, ×400.

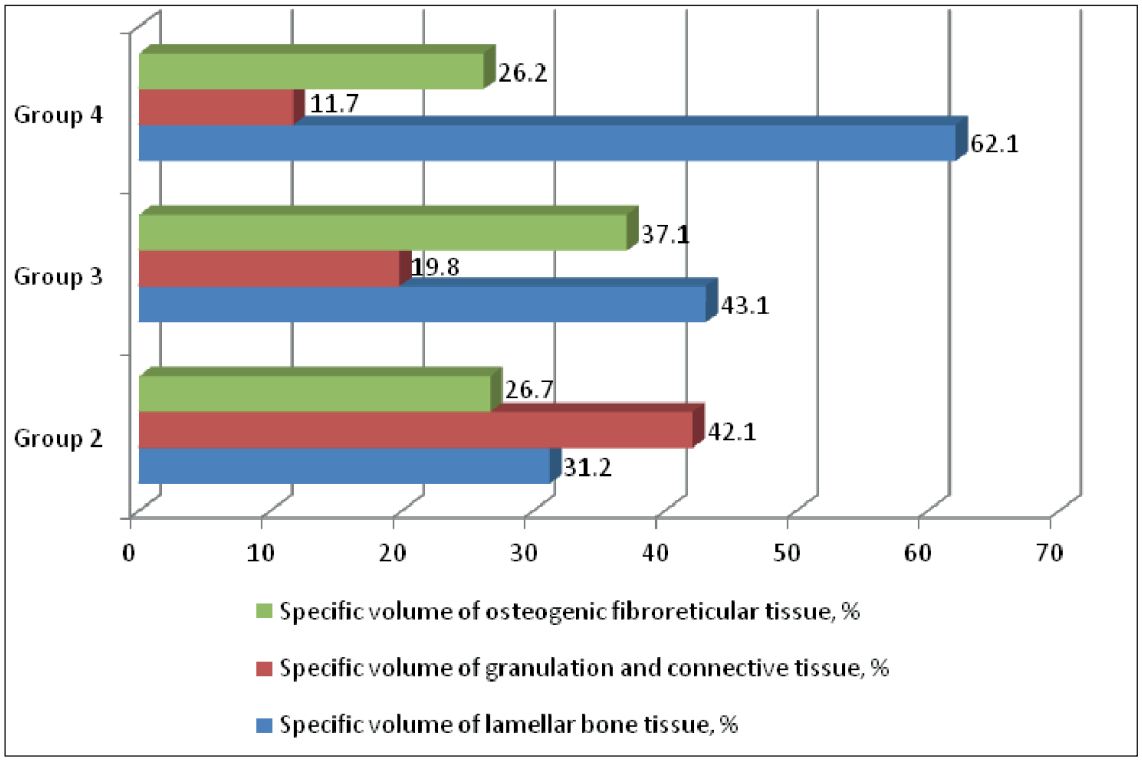

Fig. 9. The average values of specific volumes of elements (%) filling the bone cavity in the lower jaw of animals of groups 2-4 on the 14th day of the experiment.

The results of numerous studies have proven the high efficiency of the use of hydroxyapatite-containing osteotropic material for replacing bone tissue defects [13]. In the experimental and morphological study conducted by the authors, synthetic bone graft "Biomin GT" (RAPID, Ukraine) was used, which includes hydroxyapatite and β-tricalcium phosphate. According to the literature, the above-mentioned components are identical to the mineral component of bone tissue in terms of their chemical composition and mechanical properties; characterized by biocompatibility, osteoconductivity; promote osteoid mineralization; increase the proliferative potential of osteoblasts; prevent the development of inflammation;

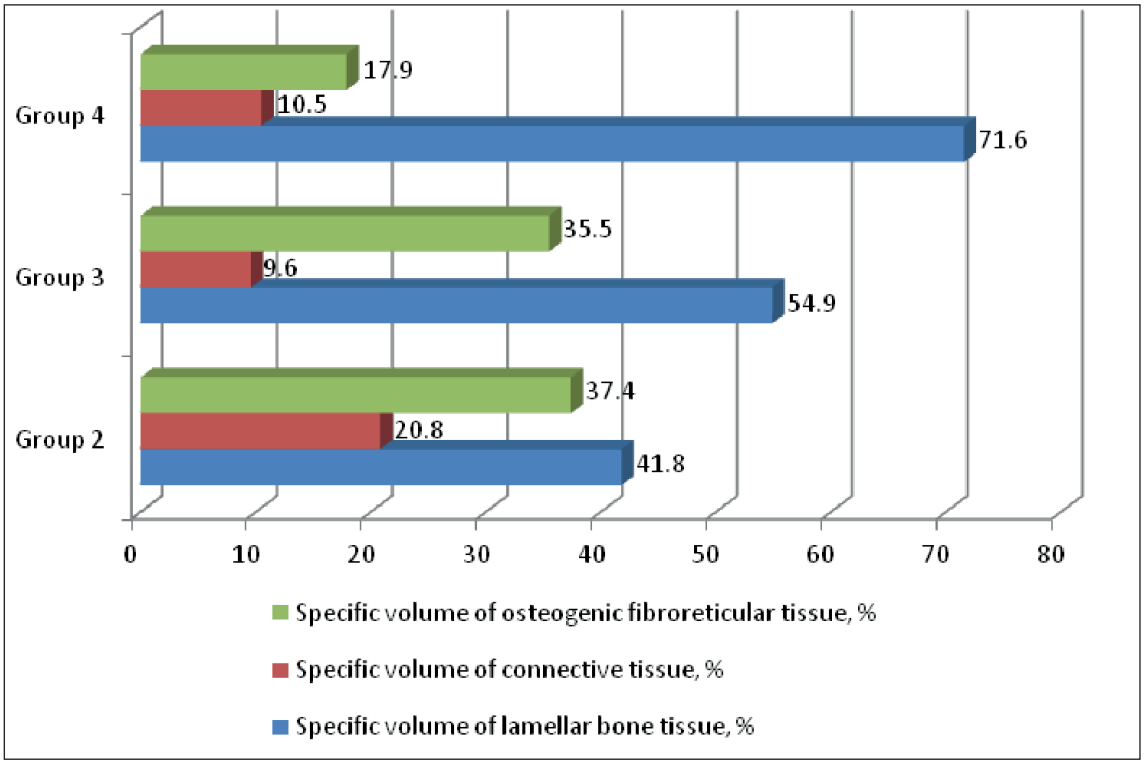

Fig. 10. The average values of specific volumes of elements (%) filling the bone cavity in the lower jaw of animals of groups 2-4 on the 28th day of the experiment.

contribute to the formation of a full-fledged bone regenerate. The research carried out by the authors also proved that the use of the above-mentioned bone replacement material helps to increase the regenerative potential of the bone tissue of the lower jaw, but does not lead to the formation of a full-fledged bone regenerate on the 28th day of the experiment, as evidenced by the results of the morphometry of the regenerate (the specific volume of lamellar bone tissue accounted for 54.9%); disordered localization of bone beams, which were characterized by reduced signs of mineralization; the presence in connective, osteogenic fibroreticular and lamellar bone tissues the encapsulated bone graft granules with the presence of inflammatory cell infiltration.

In cases the combined use of synthetic bone graft "Biomin GT" and electrical stimulation, the authors noted more intensive reparative osteogenesis processes in the bone defect of the lower jaw compared to cases when only one bone graft was used, but they also did not lead to the formation of a full-fledged bone regenerate. Numerous scientists have also noted the positive effect of electrical stimulation on healing processes, because the specified physical factor stimulates the transformation and differentiation of various cells into osteogenic cells; activates the proliferative potential of osteogenic cells; improves bone tissue trophism; activates macrophage cells, which helps to clean the bone cavity from altered cells etc. [10, 14].

CONCLUSIONS

The experimental and morphological study conducted by the authors proved that the use of hydroxyapatite-containing osteotropic material ("Biomin GT"), especially in cases of its combined use with electrical stimulation, significantly activates reparative osteogenesis in the bone defect of the lower jaw, which does not lead to the formation of a full-fledged bone regenerate.

The prospect of further research is the search of mechanisms that activate reparative osteogenesis in the bone cavity of the lower jaw in cases of using hydroxyapatite-containing osteotropic material and electrical stimulation.

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CONFLICT OF INTEREST

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FEATURES OF RHEOVASOGRAPHIC PARAMETERS OF THE TIBIA IN VOLLEYBALL PLAYERS OF A HIGH LEVEL OF SKILL OF THE YOUTH AGE

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ABSTRACT

Aim of the work was to determine the peculiarities of rheographic parameters of the tibia in volleyball players of the general group and individual somatotypes.

Materials and Methods: A comprehensive examination of 108 highly skilled volleyball players and 130 practically healthy girls aged between 16 and 20 years who did not play sports was conducted. The rheovasographic parameters of the tibia were determined using tetrapolar reocardiography on a computer diagnostic complex with the assessment of amplitude, time, and indicators of the ratio of amplitude and time rheovasographic parameters. A somatotypological study was conducted based on a calculated modification of the Heath-Carter method (1990) with the division of volleyball players and non-athletes into 4 constitutional groups: mesomorphic, ectomorphic, ecto-mesomorphic, and intermediate type.

Results: Significant differences in the value of individual amplitude and most tibia rheovasographic parameters between volleyball players and girls of the control group were revealed. In athletes, the amplitudes of the systolic, diastolic and rapid blood filling, the duration of the rheographic wave, the time of the ascending and descending parts of the rheogram and slow blood filling were statistically significantly higher. Somatotypological features of peripheral hemodynamic indicators were determined in volleyball players; the greatest differences in the indicators of regional blood circulation on the tibia were recorded for representatives of the ectomorphic somatotype;

between volleyball players and non-athletes with ecto-mesomorphic somatotype, there was no significant difference in the value of all tibia rheovasographic parameters.

Conclusion: Systematic training and competitions lead to prominent changes in rheovasographic indicators of the tibia in young female volleyball players, which are manifested by a better state of capillary and venous blood filling of the tibia muscles, but at the same time a reduced speed of regional blood flow due to a reduced tone of the vascular wall of small and medium arteries.

KEY WORDS: peripheral hemodynamics, reogravasography, tibia, somatotype, volleyball players, youth

INTRODUCTION

In modern sports, in order to achieve high results, the athlete's technical and tactical skills must be based on the perfect functioning of his or her cardiovascular system [1, 2]. Numerous scientific studies argue the importance of central hemodynamic indicators for sports performance [3, 4] and note the peculiarities of these parameters in representatives of various sports [5, 6]. Some scientists draw attention to the importance of taking into account the state of peripheral blood circulation [7-9], because the state of the vascular wall, blood supply and microcirculation of skeletal muscles, especially of the lower limb, can be a limiting factor for the successful implementation of a sports career [10]. During physical exercises, the most significant changes in blood supply are characterised for the blood circulation of skeletal muscles, therefore, the state of peripheral vessels is of great importance for the development of adaptive reactions to sports loads. The study of the physiological mechanisms of adaptation of regional blood circulation indicators in athletes of a specific sport during certain types of muscle work plays an important role both for experimental and clinical practice. Rheovasography (plethysmography) remains one of the non-invasive, simple, accessible diagnostic methods for assessing the efficiency of blood supply to the limbs and is included in the protocols of the USA and other countries of the world for screening examinations both in athletes and in the general population [11, 12].

In the practice of sports medicine, determining the state of peripheral vessels is of primary importance for indication of the pre-pathological and pathological conditions, admission to further training and competition activities, and it will allow to adequately determine the dependence of the

hemodynamic profile of the athlete and the level of his sports preparation [13]. At the same time, it is necessary to take into account the type of sport, gender and age of the athlete and his constitutional features, because there is convincing evidence of somatotypological conditioning of many morpho-functional indicators of the cardiovascular system [14 - 16].

The aim of the work was to determine the differences in the value of time, amplitude and ratios of amplitude and time rheographic parameters of the lower leg between groups of volleyball players and girls who did not play sports, within individual somatotypes and in general.

AIM

The aim of the work was to determine the peculiarities of rheographic parameters of the tibia in volleyball players of the general group and individual somatotypes.

MATERIALS AND METHODS

Based on the scientific laboratory of the National Pirogov Memorial Medical University (VNMU), we conducted an examination of 108 volleyball players between 16 and 20 years old. The average age in this group of female athletes was 18.05 ± 1.39 years. The age distribution among volleyball players was as follows: 16-year-olds – 20 persons (18.52%); 17-year-olds – 18 (16.67%); 18-year-olds – 28 (25.92%); 19-year-olds – 20 (18.52%); 22 (20.37%) of 20-year-olds. All the sportswomen had a high level of sportsmanship: 8 persons (7.41%) had 2nd adult class, 61 (56.48%) 1st adult class, 27 (25.00%) master of sports candidates, 27 (25.00%) masters of sports 12 (11.11 %). The average sports experience of female volleyball players was 6,361 ± 2,866 years.

Volleyball players were studied during the competitive period of the macrocycle, at least 12 hours after training loads. All athletes underwent a preliminary echocardiographic and electrocardiographic examination. Volleyball players who showed signs of overtraining and overstrain of the cardiovascular system were excluded from further research and were not included in the observation group. Exclusion criteria were hypertension, arrhythmias, II-III degree regurgitation, severe myocardial hypertrophy.

From the database of the scientific research center of VNMU, 130 practically healthy girls of the same age who did not play sports were selected, and they made up the control group. The average age in this group was 17.91 ± 1.49 years. The age distribution of girls in the control group was as follows: 16-year-olds - 30 persons (23.08%); 17-year-olds – 30 (23.08%); 18-year-olds – 22 (16.98%); 19-year-olds – 17 (13.08%); 20-year-olds – 31 (23.84%). The conclusion about the state of health of the girls of this group was made after a complex clinical and laboratory examination (echocardiography, sonography of the thyroid gland, kidneys, bladder, spleen, liver, gall bladder, X-ray of the chest; spirography, tetrapolar rheography. In order to decrease the influence of the hormonal state on hemodynamic parameters, we examined the girls during a period - from the 5th to the 12th day of the ovulatory-menstrual cycle, counting the day of the onset of menstruation as the first day of the cycle. The rheovasographic parameters of the tibia in volleyball players and girls of the control group were determined using tetrapolar rheocardiography on a computer diagnostic complex, and the amplitude, time and ratio indicators of the amplitude and time rheovasographic parameters were evaluated. The anthropometric study was carried out according to the method of V.V. Bunak [17], the somatotypological study - according to the calculated modification of the Heath-Carter method [18].

After somatotyping, it was established that 28 volleyball players and 33 girls of the control group belonged to the mesomorphic type of constitution, 27 girls of the first group and 36 of the second group belonged to the ectomorphic type, 27 volleyball players and 24 non-athletes belonged to the ecto-mesomorphs, and 26 volleyball players and 37 girls belonged to the medium intermediate type control group.

The analysis of the obtained results was carried out in the licensed package Statistica 5.5 using nonparametric methods of estimation of indicators.

RESULTS

We have established that the most of the amplitude rheovasographic indicators of the tibia are comparatively larger in volleyball players (Table 1). Only the value of the basic impedance and the amplitude of the incisura practically did not differ between the comparison groups. While the amplitudes of systolic and diastolic waves and rapid blood filling in volleyball players were significantly higher than in the control group (in all cases $p<0.05$).

We have established that most of the tibia amplitude rheovasographic parameters are comparatively higher in volleyball players (Table 1). Only the value of the basic impedance and the amplitude of the incisure practically did not differ between the comparison groups. While the systolic and diastolic waves amplitudes and rapid blood filling in volleyball players were significantly higher than in the control group (in all cases $p<0.05$).

In volleyball players of a high level of sportsmanship, all time indicators of the tibia rheovasogram, except for the time of rapid blood filling, were higher than in non-athletes girls (Table 1). It was found that the duration of the rheovasographic wave, the time of the ascending and descending part of the rheogram, and the slow blood filling of the tibia vessels were statistically significantly higher in volleyball players than in the control group (in all cases $p<0.001$).

We found that the integral indicators, which illustrate the ratio of the amplitude and time parameters of the tibia rheovasogram, do not reliably differ between volleyball players and non-athletes. In most cases, the average values of indicators of this group are lower in volleyball players, with the exception of the value of the diastolic index (Table 1).

In order to study the influence of the constitutional features or the specifics of sports activity, we compared the tibia rheovasographic indicators of the individuals in the same somatotype. It was found that most of the

Table 1. Indicators of the rheovasogram of the tibia in volleyball players and non-athletes (M±σ)

Radiographic indicators	Control (n=130)	Volleyball players (n=108)	p
Amplitude indicators (Om)			
Z	88.18±14.37	88.13±14.17	0.971
H1	0.055±0.0141	0.059±0.014	<0.05
H2	0.021±0.069	0.021±0.010	0.479
H3	0.022±0.010	0.024±0.008	<0.05
H4	0.023±0.006	0.025±0.007	<0.05
Time indicators (s)			
C	0.854±0.120	0.932±0.152	<0.001
A	0.139±0.018	0.148±0.023	<0.001
B	0.715±0.115	0.784±0.143	<0.001
A1	0.056±0.018	0.060±0.023	0.095
A2	0.083±0.011	0.088±0.010	<0.001
Indicators of ratios of amplitude and time parameter			
H2H1 (%)	36.95±17.36	34.22±13.49	0.665
H3H1 (%)	39.10±10.78	40.47±8.77	0.167
H4A1 (Om/s)	0.440±0.134	0.445±0.125	0.619
H1H4A2 (Om/s)	0.395±0.109	0.388±0.096	0.922
AC (%)	15.98±2.55	15.63±2.64	0.218
A1C (%)	6.058±1.961	5.931±2.112	0.250
A2C (%)	9.389±1.792	9.176±1.733	0.342
A1A2 (%)	69.34±27.84	69.29±31.79	0.587

Note: Here and later: Z – basic impedance, H1– systolic wave amplitude; H2 – amplitude of the incisure; H3 – diastolic wave amplitude; H4 – fast blood filling amplitude; C – reographic wave duration; A – time of the ascending part; B – time of the descending part; A1 – rapid blood filling time; A2 – slow blood filling time; H2H1 – dicrotic index; H3H1 – diastolic index; H4A1 – average speed of fast blood filling; H1H4A2 – average speed of slow blood filling; AC – tonus of all arteries; A1C – large diameter artery tonus indicator; A2C – indicator of arteries tonus of medium and small diameters; A1A2 – indication of the artion tones ratio.

Table 2. Amplitude and time parameters of the tibia reovasogram in individuals with mesomorphic and ectomorphic somatotypes (M±σ)

Indicators	Mesomorphic somatotype		Ectomorphic somatotype	
	Control	Volleyball players	Control	Volleyball players
Amplitude indicators (Om)				
Z	83.52±12.26	83.63±12.07	95.45±16.63	95.42±16.65
H1	0.049±0.009	0.056±0.013*	0.055±0.011	0.064±0.016*
H2	0.021±0.013	0.019±0.008	0.023±0.011	0.026±0.013
H3	0.021±0.008	0.022±0.007	0.023±0.008	0.028±0.009
H4	0.020±0.004	0.023±0.005	0.024±0.007	0.028±0.007*
Time indicators (s)				
C	0.862±0.153	0.887±0.153	0.836±0.131	0.927±0.164*
A	0.136±0.017	0.148±0.023*	0.141±0.019	0.149±0.027
B	0.726±0.151	0.738±0.142	0.695±0.122	0.778±0.146*
A1	0.055±0.015	0.057±0.023	0.061±0.019	0.064±0.029
A2	0.081±0.011	0.091±0.013**	0.080±0.010	0.085±0.009**

*Note: Here and later: * – p<0,05; ** – p<0,01;*

Table 3. Indicators of the ratios of amplitude and time parameters of the tibia reovasogram in individuals with mesomorphic and ectomorphic somatotypes (M±σ)

Indicators	Mesomorphic somatotype		Ectomorphic somatotype	
	Control	Volleyball players	Control	Volleyball players
H2H1 (%)	40.65±21.81	33.711±10.84	40.47±17.20	38.88±16.18
H3H1 (%)	40.88±11.60	38.29±6.00	41.05±11.48	43.00±11.00
H4A1 (Om/s)	0.396±0.119	0.421±0.114	0.427±0.089	0.481±0.146
H1H4A2 (Om/s)	0.355±0.082	0.363±0.093	0.391±0.076	0.421±0.111
AC (%)	15.63±3.09	16.56±2.64	16.60±2.59	15.74±2.35
A1C (%)	5.961±1.549	6.00±2.13	6.783±2.136	6.320±2.203
A2C (%)	9.192±2.227	9.962±1.989	9.333±1.816	8.940±1.679
A1A2 (%)	69.61±22.74	65.02±30.75	77.60±29.14	77.52±42.04

Table 4. Amplitude and time parameters of the tibia reovasogram in patients with ecto-mesomorphic and medium intermediate somatotypes (M±σ)

Indicators	Ecto-mesomorphic somatotype		Medium intermediate somatotype	
	Control	Volleyball players	Control	Volleyball players
		Amplitude indicators (Om)		
Z	84.62±10.22	88.42±12.71	90.95±14.92	86.87±13.71
H1	0.059±0.012	0.059±0.015	0.061±0.019	0.057±0.014
H2	0.016±0.003	0.019±0.008	0.024±0.015	0.019±0.013
H3	0.020±0.004	0.024±0.007	0.026±0.014	0.025±0.009
H4	0.023±0.005	0.025±0.007	0.025±0.008	0.024±0.009
		Time indicators (s)		
C	0.861±0.114	0.942±0.149	0.864±0.106	0.962±0.134*
A	0.139±0.009	0.146±0.019	0.137±0.018	0.146±0.027
B	0.722±0.116	0.796±0.145	0.727±0.103	0.816±0.126*
A1	0.052±0.012	0.060±0.020	0.053±0.016	0.057±0.024
A2	0.087±0.012	0.086±0.008	0.084±0.010	0.089±0.010*

amplitude indicators of the tibia did not have significant differences between non-athletes and volleyball players of the mesomorphic somatotype. Only the systolic wave amplitude in the group of female athletes was higher ($p<0.05$) than in the control group (Table 2). The average values of most amplitude indicators, with the exception of the amplitude of the incisure, are higher in mesomorph volleyball players.

In sportswomen with an ectomorphic somatotype, the average values of the amplitude indicators of the tibia rheovasogram, with the exception of the value of the basic impedance, are larger than in the girls-ectomorphs of the control group. Statistically significant differences were established for the systolic wave amplitudes and rapid blood filling (in both cases $p<0.05$) (Table 2).

We established that the time of the ascending part of the rheovasogram ($p<0.05$) and the slow blood filling of the tibia vessels ($p<0.01$) were significantly longer in volleyball players of the mesomorphic somatotype than in girls who did not play sports of the same somatotype (Table 2). Other time indicators had larger average group values, although the difference was not reliable when compared. Representatives of the ectomorphic somatotype had significant differences in the value of the time indicators of the tibia rheovasogram. It was found that in volleyball players of this somatotype, the duration of the rheovasographic wave ($p<0.05$), the time of the descending part ($p<0.05$) and slow blood filling ($p<0.01$) of the tibia rheovasogram are higher with the compared control group (Table 2).

It was established that the ratios of the amplitude and time parameters of the tibia rheovasogram in volleyball players and girls of the control group with mesomorphic and ectomorphic somatotypes did not differ statistically significantly (Table 3).

We did not determine a reliable difference when comparing the amplitude and time indicators of the tibia rheovasogram in volleyball players and girls of the control group with ecto-mesomorphic somatotype, although it should be noted that the athletes had higher average group values of these parameters (Table 4).

In volleyball players with an intermediate somatotype, all amplitude indicators are higher than in controls, but no significant differences were indicated. The time indicators of the tibia rheovasogram had a significant difference

Table 5. Ratios of amplitude and time parameters of the tibia reovasogram in individuals with ecto-mesomorphic and medium intermediate somatotypes (M±σ)

Indicators	Ecto-mesomorphic somatotype		Medium intermediate somatotype	
	Control	Volleyball players	Control	Volleyball players
H2H1 (%)	27.31±5.91	33.43±13.42	36.53±14.29	32.16±13.98
H3H1 (%)	34.54±7.37	39.40±8.97	41.21±11.48	42.32±8.35
H4A1 (Om/s)	0.462±0.105	0.446±0.137	0.493±0.169	0.437±0.101
H1H4A2 (Om/s)	0.419±0.091	0.400±0.097	0.430±0.146	0.371±0.081
AC (%)	15.92±2.59	15.32±2.67	15.58±2.39	14.86±2.49
A1C (%)	5.538±1.534	5.983±2.057	5.652±1.885	5.386±2.198
A2C (%)	9.731±2.204	8.833±1.434	9.303±1.479	8.932±1.391
A1A2 (%)	62.73±23.26	70.58±27.96	64.56±22.85	64.29±26.34

between these comparison groups. The duration of the rheographic wave, the time of the descending part of the rheovasogram and the slow blood filling of the tibia vessels were significantly larger in volleyball players of intermediate somatotype compared to non-athletes girls of the same somatotype (in all cases $p<0.05$). In addition, there was a tendency of increasing the time of the ascending part of the rheographic wave and rapid blood filling in volleyball players of this constitutional type (Table 4).

It was established that the indicators of the ratio of the amplitude and time parameters of the tibia rheovasogram in volleyball players and girls of the control group with ecto-mesomorphic and intermediate somatotypes did not differ statistically significantly (Table 5).

But it should be noted that ecto-mesomorph volleyball players compared to non-athletes of the same somatotype had higher average statistical values of dicrotic index and diastolic index, indicators of the tone of large-diameter arteries and the ratio of arterial tones. And the tone index of all arteries, arteries of medium and small diameters, the average speeds of slow and fast blood filling in them were lower. In volleyball players with an intermediate somatotype, the large number of the integral indicators of the tibia rheovasogram were lower than in girls of the control group (Table 5).

DISCUSSION

Modern volleyball is one of the most popular sports both in Ukraine and in the world. During one game, each athlete performs a large number of technical tricks, about 200 jumps, a huge number of throws, high-speed movements on the sports field with a sharp change in direction, sudden stops and falls. During a volleyball game, the heart rate can increase to 200-220 beats per minute. Therefore, it is clear that modern volleyball makes high demands on the physical development of the volleyball player, his speed and strength training, and requires special endurance, which is limited by the state of the cardiovascular system [3, 19], in particular, central hemodynamics [5, 6, 20]. Studying a group of teenage volleyball players, Yu.I. Yakusheva [4] determined that their stroke and minute volumes, stroke index, volume spreed of blood movement and left ventricular power are significantly higher, and total peripheral resistance is significantly lower compared to girls of the same age who do not do sports.

The volume of blood flow in the arteries of the lower extremities is adjusted to the metabolic needs of the special muscles. In the studies of O.P. Khapitska et al. [8] were proved that sports specialization has influence to the peculiarities of muscle activity and led to marked differences in the parameters of peripheral hemodynamics between volleyball players, athletes, and wrestlers. In the group of young volleyball players, compared to athletes of the same age, the following rheovasographic indicators of the hip were significantly lower: the time of the descending part of the rheogram and the rheographic wave, while the time of the ascending part and slow blood filling of the rheovasogram, basic impedance, amplitude of the incision, and indicators of arterial tone were statistically significantly greater [7]. Indicators of local tibia blood flow also had a significant difference between young athletes of various sports. In particular, the time of the rheographic wave and the descending part of the rheogram was shorter in volleyball players compared to the athletes, and the amplitude parameters were larger: basic impedance, systolic wave amplitudes and rapid blood filling, the tone index of all arteries, the duration of the ascending part and slow blood filling of the tibia rheogram [8, 9].

Analyzing our results, it should be noted that in general group of volleyball players, compared to girls who did not play sports, is indicated higher amplitude and time rheovasographic indicators of the tibia. We found that the amplitude of the systolic wave, which indirectly indicates the functional state of the vessels and depends on a sufficiently large number of factors: heart rate, systolic blood volume, blood pressure and the tone of the vascular walls, was significantly higher in volleyball players compared to the control group [21].

Predominantly, when vascular tone increases, the amplitude of the systolic wave decreases, when vascular tone decreases - it increases. In addition, the amplitude of the diastolic wave, which reflects the ratio of arterial and venous blood flow, was also greater in volleyball players [22]. With the elasticity of arterial vessels, it is mainly a reflection wave

from the smallest arteries and arterioles. Since the degree of this reflection is functionally connected through the venular-arteriolar reflex with the state of venous full blood of the organ, it indirectly depends on the state of venous outflow [23]. The amplitude of rapid blood filling, which depends on the elasticity and tone of vascular walls [8, 21], is also higher in volleyball players.

Thus, it can be concluded that the amplitude parameters of the rheovasogram in volleyball players depend on many factors, in particular, on the work of the heart itself and the state of the vascular bed. In volleyball players, the state of capillary and venous blood supply of the tibia muscles is better compared to girls who do not play sports, but at the same time, they have a reduced tone of the arterial part of the vascular bed, which is confirmed by an increase in the amplitude indicators of the rheovasogram. Athletes who played volleyball showed a slight blood spreed, which is confirmed by their significantly higher values of the duration of the rheographic wave, the time of the ascending and descending parts of the rheogram, and slow blood filling. The time of the ascending part is the most stable indicator of the rheogram and does not depend on the heart rate, but reflects the period of complete opening of the vessel and provides clear information about the state of the vascular wall, because it depends on the tone of resistance vessels (arterioles and capillaries) [8, 23]. The time of the descending part depends, first of all, on the frequency of heart contractions, and its increase is also caused by a decrease in the elasticity of the vascular wall. The value of the time of slow blood filling is mainly determined by the tonic properties of the vascular wall of small and medium arteries. Therefore, based on the results of the analysis of the tibia indicators of the rheovasogram of the lower leg, it can be concluded that the elasticity of the vascular bed is somewhat reduced in volleyball players, which leads to the lengthening of the temporal characteristics of the rheovasogram.

At the same time, we did not find a reliable increase in arterial tone indicators in volleyball players. This can also be explained by the adaptive propeties of the female body, because all indicators of arterial tone, the time of slow blood filling and the ascending part of the rheovasogram of the lower leg tibia were statistically significantly higher in male representatives who were professionally involved in volleyball [7, 8, 9], and lower dicrotic index, time of the rheographic wave and the descending part of the rheogram compared to non-athletes.

The authors explain the detected changes with a pronounced slowing of blood flow in the tibia, a decrease in the elasticity of the vascular walls of arteries of medium and small diameters, high peripheral resistance, an increase in the tone of arteries of different diameters, which can have negative consequences for the health of athletes, because they can be signs of phlebopathy, which manifested by symptoms of venous stasis without revealing pronounced signs of organic pathology of the venous system [24].

The determination of individual indicators of the cardiovascular system by anthropo-somatotypological characteristics of the human body has been proven by many studies [14, 15, 16, 25, 26]. Indicators of regional blood flow of the lower extremities are related to the anatomical variability of the hip and lower leg in representatives of different sexes and body types [27]. We also discovered somatotypological features of rheovasographic indicators of the tibia in volleyball players. Within individual constitutional types, not all the differences we found between the general comparison groups in regional blood circulation indicators were preserved.

In particular, there was no significant difference between volleyball players and non-athletes with ecto-mesomorphic somatotype in the value of all rheovasographic parameters of the tibia; in volleyball players of mesomorphic somatotype, only 3 rheovasographic indicators were significantly higher than in girls who did not play sports of the same somatotype: the systolic wave amplitude, the time of the ascending part and slow blood filling. In volleyball players with an intermediate somatotype, the duration of the rheographic wave, the time of the descending part of the rheovasogram and slow blood filling of the tibia vessels were significantly higher.

The greatest differences in the indicators of tibia regional blood circulation were in representatives of the ectomorphic somatotype, who were characterized by significant longitudinal body dimensions and small muscle volumes and minimal development of subcutaneous fat deposits. In ectomorph volleyball players, compared to the control group, the amplitude of the systolic wave and rapid blood filling, the duration of the rheovasographic wave, the time of the descending part and slow blood filling of the leg rheovasogram were significantly higher than in control group.

In the studies of O.P. Khapitskaya found that the most prominent differences in the value of rheovasographic parameters are between young non-athletes and volleyball players with a mesomorphic somatotype [14].

That is why, the determination of the features of peripheral hemodynamic indicators of individual somatotypes in female volleyball athletes will enable a more correct determination of the reference values of rheovasographic parameters of the tibia and focus more attention of sports physicians during medical check-ups specifically on female athletes with an ectomorphic constitutional type, because they may have the highest risk of pre-existing conditions of venous circulation pathologies, because they may have the highest risk of pre-existing conditions of venous circulation pathologies.

CONCLUSIONS

1. In the general group of volleyball players, compared to non--athletes girls, higher amplitude and time rheovasographic indicators of the tibia were found. Significant differences between the comparison groups were determined for the amplitudes of the systolic and diastolic waves and rapid blood supply, the duration of the rheographic wave, the time of the ascending and descending parts of the rheogram and slow blood filling.
2. In volleyball players with a mesomorphic somatotype, the amplitude of the systolic wave, the time of the

ascending part of the rheovasogram and the slow blood filling of the tibia vessels were significantly greater than in to non-athletes girls of the same somatotype.
3. It was established that in volleyball players of ectomorphic constitutional type, the amplitude of the systolic wave and rapid blood filling, the duration of the reovasographic wave, the time of the descending part and slow blood filling of the tibia reovasogram were statistically significantly greater compared to the control group.
4. No significant difference was found in the value of all rheovasographic parameters of the tibia between volleyball players and non-athletes with ecto-mesomorphic somatotype.
5. In volleyball players with an intermediate somatotype, the duration of the rheographic wave, the time of the descending part of the rheovasogram, and the slow blood filling of the tibia vessels were significantly longer in time than in controls.
6. It was established that the ratio of amplitude and time parameters of the rheovasogram of the tibia does not reliably differ between volleyball players and non-athletes of general groups and within individual somatotypes.

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GENDER AND AGE ASPECTS IN THE PATHOGENESIS OF BONE MINERAL DENSITY DISORDERS

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ABSTRACT

The aim: To assess the structural and functional state of bone tissue in terms of gender and age.

Materials and Methods: 108 people aged 54.7±14.8 years, who were divided into two groups according to gender, participated in the retrospective cohort study. All patients underwent ultrasound densitometry to assess bone mineral density (BMD) on the radial bone with an assessment of T-score, Z-score, and speed of ultrasonic wave propagation (SoS).

Results: The study found that T-score and Z-score values, which corresponded to osteoporosis, were recorded in the age group > 50 years, regardless of the gender of the subjects. All women older than 35 years had a decrease in BMD below -1.0 SD by T-score. It was determined that osteoporosis criteria clearly prevail in women of the > 50-year-old group. In osteoporosis, the SoS is significantly lower than in individuals with normal indicators of the T-criterion. According to the results of the T-score comparison, BMD disorders were determined in postmenopausal women which emphasize the importance of the level of female sex hormones in the formation of osteopenic syndrome, in contrast to men, in whom no changes in the state of bone tissue were recorded.

Conclusions: The results of the research prove the prevalence of osteopenic syndrome in terms of age and gender, with an emphasis on women aged 50 years and older. Screening for BMD disorders using ultrasound densitometry is appropriate and allows taking measures to prevent the progression of osteoporosis in the early stages.

KEY WORDS: osteoporosis, osteopenia, bone mineral density, ultrasound densitometry, gender and age aspects, postmenopause

INTRODUCTION

Osteoporosis is a systemic progressive disease of the musculoskeletal system, caused by the loss of bone mass with the disrupted microarchitectonics of bone tissue, which leads to an increase in bone fragility and the risk of fractures [1]. This disease is observed in all age groups, regardless of gender and race, but the highest prevalence is observed in Caucasians (white race), postmenopausal women, and the elderly. With an aging population and a decrease in life expectancy, osteoporosis is increasingly becoming a global epidemic [2, 3]. Currently, it is estimated that more than 200 million people suffer from osteoporosis [3, 4]. According to the latest statistics from the International Osteoporosis Foundation, 1 in 3 women over 50 years of age and 1 in 5 men worldwide experience osteoporotic fractures during their lifetime [5]. Osteoporosis is a significant risk factor for fractures in the same way that hypertension is a risk factor for stroke [4]. According to estimates, it has been established that 40% of women suffer from osteoporosis, of which 30% are postmenopausal women. 30% of men experience osteoporotic fractures during their lifetime [6]. According to the International Osteoporosis Foundation (IOF), the number of postmenopausal women in Ukraine with osteoporosis and osteopenia is 7 million (28% of the total number of women) [7]. Today, about 2.5 million women and 900.000 men suffer from osteoporosis in Ukraine, 50% of osteoporosis patients become disabled as a result of the disease, and 20% die. According to the prognosis, by 2050, the number of hip fracture cases among men will increase by 310%, and among women – by 240%, compared to the indicators as of 1990 [8].

An imbalance between the formation of bone tissue and the speed of its resorption, where the latter prevails, underlies the development of osteoporosis. Modeling of bone tissue occurs from birth to adulthood, it reaches its peak during puberty, and is called peak bone mass (PBM). PBM is determined by genetic factors, the balance of sex hormones, the state of health during the development of bone tissue, concomitant pathology, and taking medications (in particular, glucocorticoids, cytostatics, and anticonvulsants) [9].

Glucocorticoids are used to treat a wide range of diseases, and an estimated 1-2% of the population receives long-term glucocorticosteroid therapy [8, 10]. Glucocorticoids affect bone resorption by increasing the production of macrophage colony-stimulating factor (M-CSF) and RANKL, which leads to a decrease in the production of osteoprotegerin (OPG) by osteoblastic cells and osteocytes, and, as a result, an increase in both the number and activity of osteoclasts [11-14].

The indisputable cause of the formation of osteoporosis is considered to be a deficiency of estrogens – the main hormonal regulators of bone resorption. They have an

osteoprotective effect on bone tissue, acting through specific receptors or local mediators, starting a cascade of growth and differentiation of bone tissue cells.

Densitometry is considered the gold standard in the diagnosis of osteoporosis. Densitometry is an equipment-based non-invasive method of determining bone mineral density (BMD), whose main task is to detect osteoporosis in the early stages of development. There are several types of densitometry: quantitative ultrasonic densitometry (QDM), dual-energy X-ray absorptiometry (DRA or DXA) and quantitative computer tomography [15].

Along with ultrasonic (US) densitometry, the FRAX scale is used. FRAX is a computer algorithm created in 2008 by the World Health Organization (WHO) Collaborating Center in Sheffield. The FRAX instrument consists of seven dichotomous clinical risk factors that include previous fractures, parental hip fractures, smoking, systemic glucocorticoid use, excessive alcohol consumption, rheumatoid arthritis, and other causes of secondary osteoporosis. In addition to age, gender, and body mass index (BMI), these risk factors contribute to the estimation of the 10-year probability of fracture, regardless of bone mineral density (BMD) [16, 17].

The WHO criteria for osteoporosis are based on BMD values compared to a control population of healthy young people. According to this, two main criteria are distinguished: T-score and Z-score. The diagnosis of osteoporosis is made if BMD values are below the average peak bone mass by no more than 2.5 SD (standard deviation) (T ≤ -2.5). Osteopenia (low bone mass) is verified if BMD values are more than 1 SD below the mean peak bone mass, but not more than 2.5 SD (-2.5 T ≤ -1). Depending on the T-score distribution, 3 degrees of osteopenia are distinguished: I degree (T ≤ -1.0 – > -1.5), II degree (T ≤ -1.5 – > -2.0); III degree (T ≤ – 2.0 – > -2.5). Normal values of BMD correspond to a range of more than 1 SD from the average value of peak bone mass of a young adult (T> -1) [18].

Using the method of ultrasound densitometry with a screening purpose in combination with the FRAX scale, whose functioning consists in calculating the individual 10-year probability of damage to the hip joint and large osteoporotic fractures, makes it possible to establish the state of bone tissue in the early stages of the formation of the disease, depending on gender, and predict and prevent progression of complications [19].

THE AIM

The aim of the research is the assessment of the structural and functional state of bone tissue in terms of gender and age.

MATERIALS AND METHODS

This study is a part of the initiative research project of the Department of Propaedeutics of Internal Medicine "Peculiarities of the course of cardiovascular pathology in patients of different age categories depending on the presence of components of the metabolic syndrome and comorbid conditions, the ways of correcting the detected disorders and prevention", state registration No. 0119U1028.

We conducted a retrospective cohort study. The object of the research is bone mineral density according to ultrasound densitometry data. Inclusion criteria: women (of reproductive and postmenopausal age) and men. Exclusion criteria: diabetes, rheumatic diseases, oncological diseases, including in the anamnesis, diseases of the endocrine glands, heart defects, chronic kidney and liver failure, and diseases of the blood system.

A total of 108 people aged 54.7±14.8 years, who were divided into two groups according to gender, took part in the study. The study group, which consisted of women, in turn, was divided into a subgroup of postmenopausal age – IA (74% of women) and reproductive age IB (26% of women). Group II (men) made up 8% of all studied subjects (Fig. 2). The average age of women of reproductive age (subgroup IB) was 36.2±7.0; postmenopausal women (subgroup IA) – 62.2±8.5; men – 48.5±22.7. As to the age, the distribution was carried out according to the criterion:

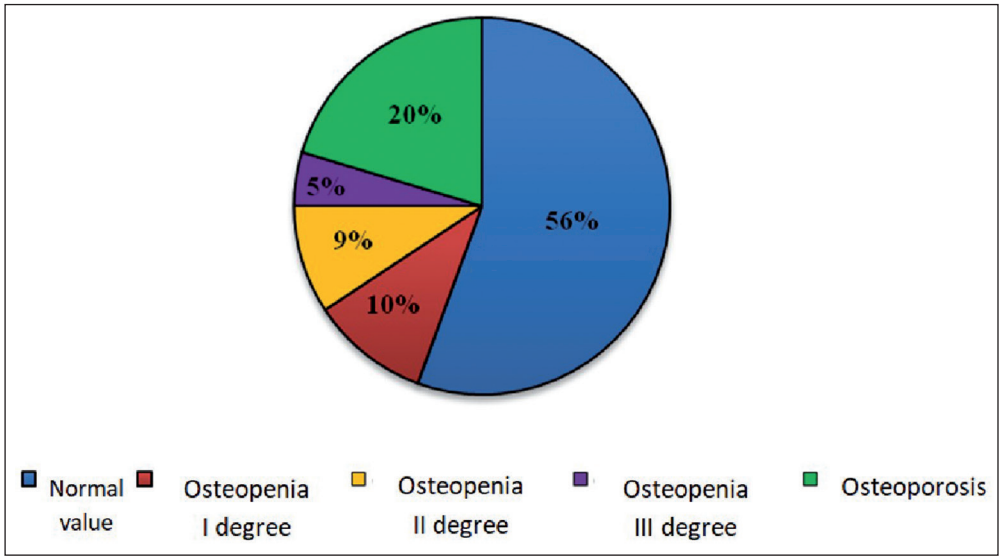


Fig. 1. Percentage distribution of patients according to the frequency of detection of abnormalities in bone mineral density (BMD).

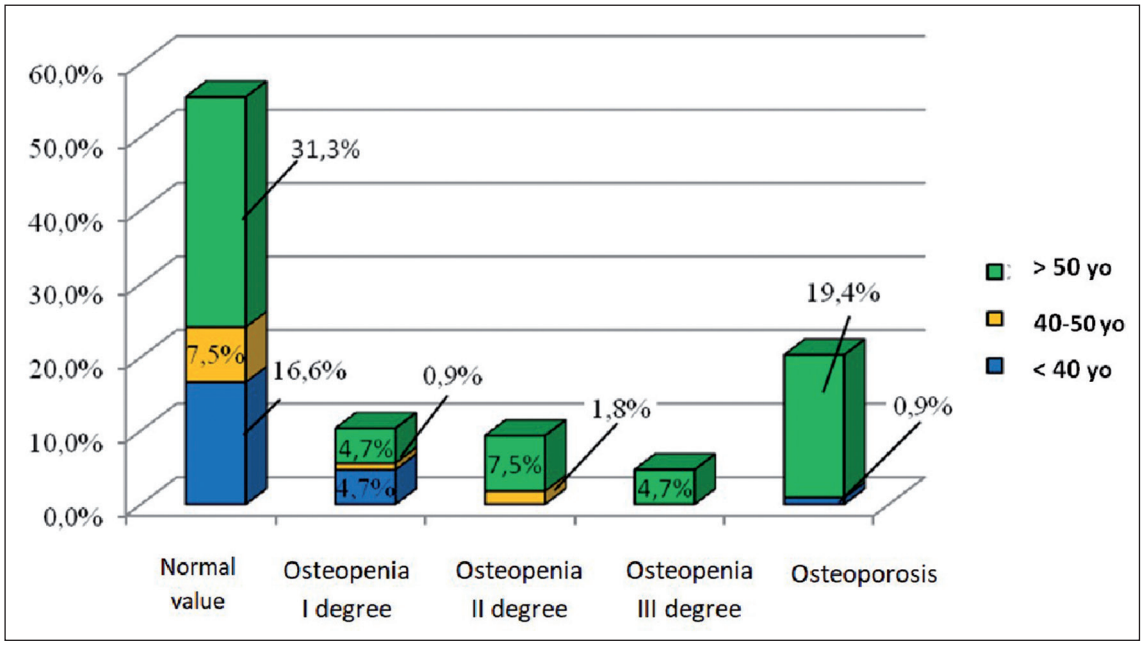


Fig. 2. Distribution by indicators of the mineral density of bone tissue in the age aspect.

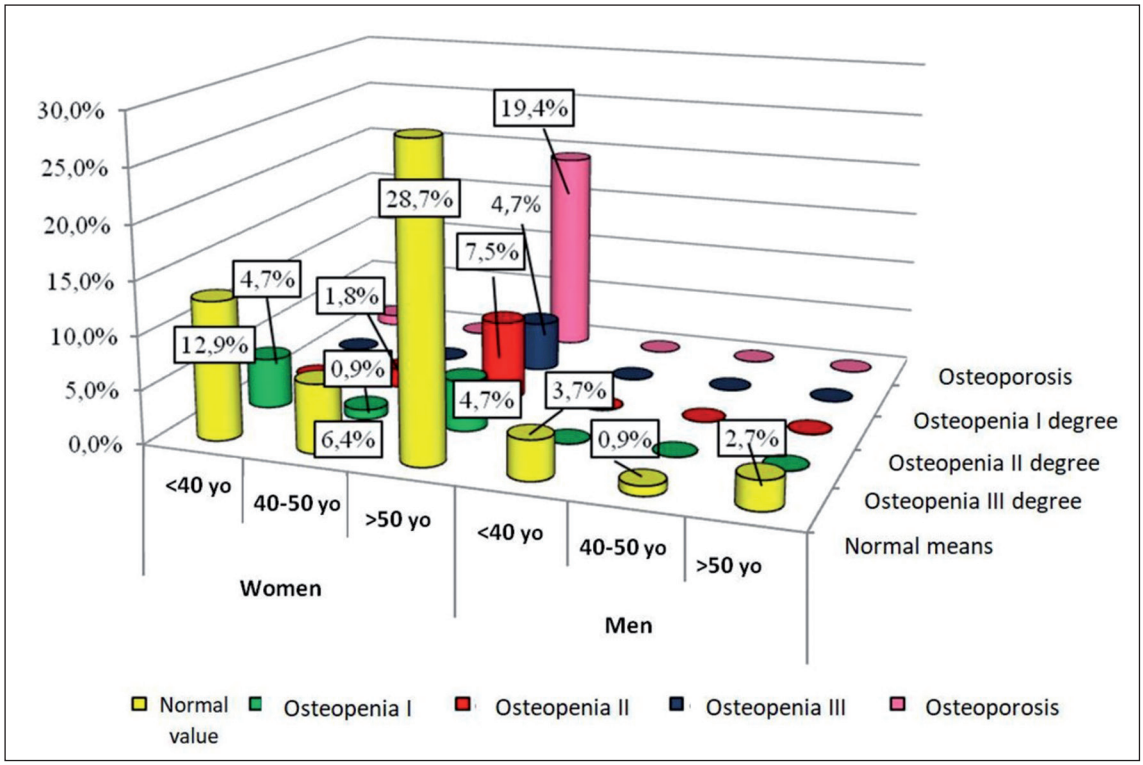


Fig. 3. Features of the distribution of BMD indicators according to age and gender.

< 40 years – 22.2% of the studied persons, 40-50 years – 11% of persons, > 50 years – 67.6% of persons.

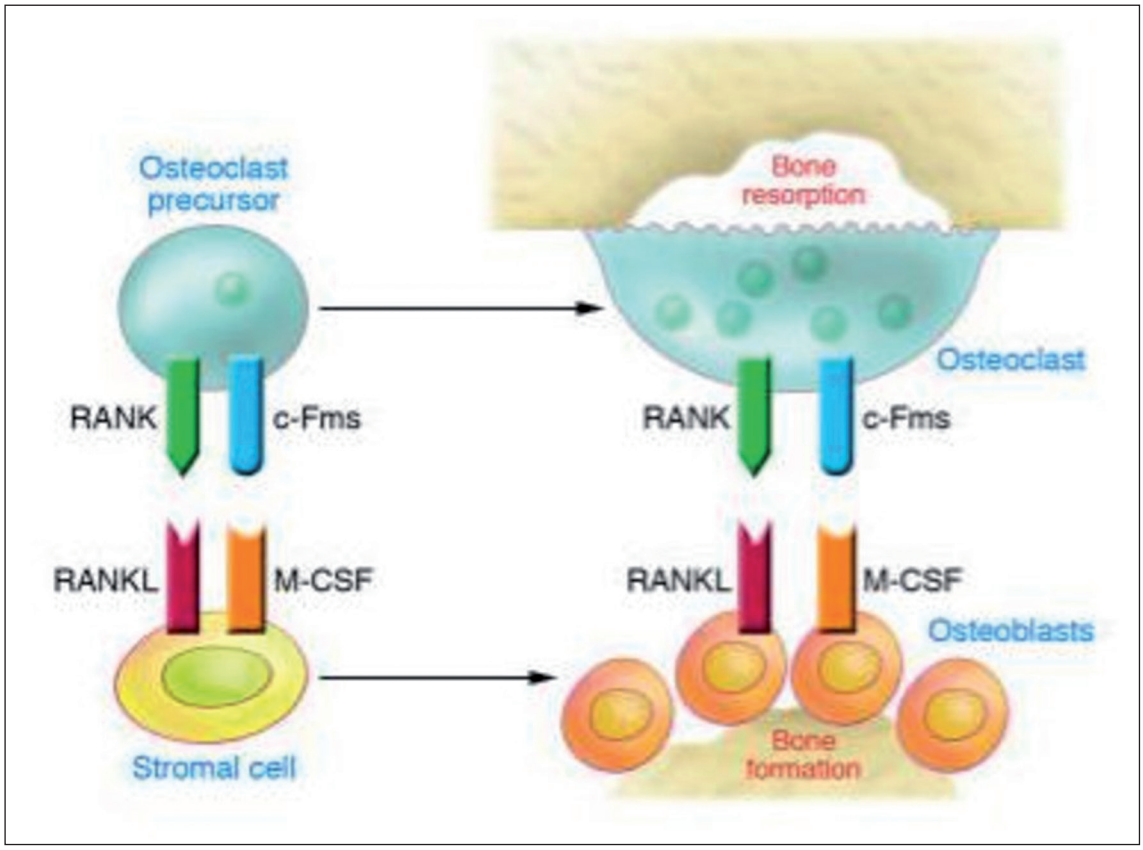
All patients underwent ultrasound densitometry to assess the structural and functional state of bone tissue. Determination of BMD was performed using an ultrasonic bone densitometer Sunlight MiniOmni on the radial bone.

T-score (T-criterion) was determined by comparing the obtained results of bone tissue density with the normal average "peak" bone mass for an adult.

Z-score (Z-criterion) was obtained by comparing the density of bone tissue with the average indicator of the norm in a given age group.

In addition, the speed of ultrasonic wave propagation (SoS) in m/sec was determined. In compliance with the T-criterion of BMD, according to WHO indicators, the character of the bone tissue structure disruption was determined.

Before conducting the study, all patients signed an informed voluntary consent to participate in this study. The research



Notes: RANK – nuclear factor κB receptor activator; C-fms – a specific receptor for M-CSF.

Fig. 4. The role of nuclear factor κB, RANKL and macrophage colony-stimulating factor (M-CSF) in the differentiation and regulation of osteoblastic activity under physiological conditions (adapted from M.N. Weitzmann, 2006 [26]).

Table 1. Indicators of the ultrasound wave propagation speed

Distribution according to the T-score/(M±σ)	Study group IA	Study group IB	Study group II
Normal value	4181.3±119.7	4128.6±16.7	4124.1±67.2
Osteopenia I degree	4036.1±23.7	4067.4±116.5	–
Osteopenia II degree	3980.2±33.2	–	–
Osteopenia III degree	3916.3±64.6	–	–
Osteoporosis	3832.5±109.9*	–	–

Notes: M – the sample mean, σ – standard quadratic deviation.
** – p <0.05 comparing study group IA with osteoporosis and groups IB and II with normal indices.*

Table 2. Indicators of bone mineral density of the radial bone according to ultrasound densitometry data

	Study group IA	Study group IB	Study group II
T-score (M±σ)	-1.55±0.29	-0.48±0.14*	0.30±0.17*
Z-score (M±σ)	-0.38±0.26	-0.21±0.13	0.56±0.15

Notes: M – the sample mean, σ – standard quadratic deviation.
** – p <0.05 significant differences in BMD in comparison with group IA.*

was conducted in accordance with the requirements of the Helsinki Declaration of 1975 and the Order of the Ministry of Healthcare of Ukraine No. 690 of 23.09.2009 "On approval of the Procedure for conducting clinical trials of medicinal products and examination of clinical trial materials".

Statistical processing of the results was carried out using KyPlot 6.0 and Microsoft Excel software. The hypothesis about the normality of the distribution was tested using the Shapiro-Wilk test. The belonging of the samples to the same population was determined using the Kruskel-

Table 3. The value of mineral density of the bone tissue of the radial bone according to the data of ultrasound densitometry by the T-criterion.

	Normal value (M±σ)	Osteopenia I degree (M±σ)	Osteopenia II degree (M±σ)	Osteopenia III degree (M±σ)	Osteoporosis (M±σ)
Study group IA	0.25±1.16	-1.12±0.13	-1.68±0.09	-2.18±0.13	-4.31±2.7
Study group IB	-0.08±0.43	-1.26±0.2	-1.7±0.14	–	–
Study group II	0.30±0.50	–	–	–	–

Notes: M – the sample mean, σ – standard quadratic deviation.

Table 4. The value of the mineral density of bone tissue of the radial bone according to the data of ultrasound densitometry by the Z-criterion.

	Normal value (M±σ)	Osteopenia I degree (M±σ)	Osteopenia II degree (M±σ)	Osteopenia III degree (M±σ)	Osteoporosis (M±σ)
Study group IA	0.8±1.06	-0.8±0.18	-0.17±0.53	-0.48±0.55	-2.36±3.13
Study group IB	0.15±0.42	-0.85±0.13	-1.7±0.14		-
Study group II	0.56±0.44	-	-	-	-

Notes: M – the sample mean, σ – standard quadratic deviation.

Wallis rank test. Data were presented as arithmetic mean and standard deviation (M+σ). Independent samples were compared using Student's t-test. Statistical significance was established under the condition that the level of statistical significance p was less than 0.05.

RESULTS

During the study, regardless of age and gender, part of the examinees (56%) had normal BMD indicators, osteopenia was registered in 24% of people: I degree – in 10%, II degree – 9%, III degree – in 5% of the examined subjects (Fig. 1). Osteoporosis was determined in 20% of the subjects.

In the group demarcated by the age criterion of < 40 years, the share of examinees who corresponded to normal T-score values was 16.6%, in the group of 40-50 years – 7.5%, in the group > 50 years – 31.3% (Fig. 1).

In addition, it was found that all women older than 35 years had a decrease in BMD below -1.0.

Values corresponding to osteoporosis were recorded in the age group > 50 years and accounted for 19.4% of the studied cohort, which is probably due to changes in the hormonal status of the subjects (Fig. 2).

Comparing the gender characteristics of the BMD of the examined subjects, we found that the criteria of osteoporosis according to the T-score clearly prevail in women of the group > 50 years old, which is shown in Figure 3.

Evaluating the speed of ultrasonic wave propagation (SoS) in m/sec, we found that in osteoporosis the value of this indicator is significantly lower than in individuals of all studied groups with normal indicators of the T-criterion ($p<0.05$) (Table 1)

According to the results of the T-score comparison, BMD disorders were observed in women of study groups IA and IB, which emphasizes the importance of the level of female sex hormones in the formation of osteopenic syndrome, unlike men (group II), in whom no changes in the state of bone tissue were recorded (Tables 2, 3, 4).

DISCUSSION

According to the data of the conducted research, the most vulnerable category in terms of BMD disorders are women in the perimenopausal, and especially, postmenopausal period. The decisive role of estrogens in relation to the state of bone tissue relies on ligand-dependent mechanisms that mediate an increase in the functional activity of osteoblasts and osteocytes, as well as in the inhibitory effect on osteoclastogenesis by activating osteoblast apoptosis [20]. Since osteoclasts are of monocyte-macrophage origin, they are specialized cells of the immune system in bone tissue and have phagocytic activity. Taking an active part in the immune response, they are capable of secreting pro-inflammatory cytokines (IL-1, IL-6, IL-17 and others), which increase the resorption of bone tissue [21].

The central role in the regulation and differentiation of osteoclasts belongs to such cytokines (CK) as the precursor of macrophage colony-stimulating factor (M-CSF) and the ligand-activator of the receptor of nuclear factor kappa B (NF-kB) – RANKL. M-CSF is an effective stimulator of RANK receptor expression and osteoclast proliferation, it also regulates apoptosis, increasing the viability of these cells [22, 23].

RANKL belongs to the family of tumor necrosis factor α (TNF-α), it is expressed by osteoblasts, stromal cells and activated immune cells. Its activation leads to the expression of the RANK receptor on osteoblasts and their progenitors. The effect of the formed RANKL-RANK complex on cell activity is carried out through signaling pathways involving NF-kB, the early response gene (c-Fos) and the transcriptional nuclear factor of activated T cells c1 (NFATc1) [24, 25].

In the RANKL-RANK-OPG system, osteoprotegerin (OPG) belongs to the CK of the TNF superfamily, it provides a protective role in bone tissue resorption and osteoclast activation, being a soluble "trap receptor" for RANKL [26] (Fig. 4).

It has been proven that estrogens can suppress the production of RANKL and M-CSF, as well as stimulate the synthesis of OPG, while under the conditions of hypoestrogenia caused by menopause, these mechanisms work in the opposite direction. Since the expression of RANKL is controlled by estrogens, the lack of formation of the corresponding hormones leads to an increase in the concentration of RANKL due to the activation of cells of the immune system, which is one of the mechanisms of postmenopausal osteoporosis and it occupies one of the leading links in the development of this pathology.

Women in the period of early hypoestrogenia lose about 5-15% of bone tissue, which affects the quality and length of life with the mediated formation of comorbid pathology. The relationship between hypoestrogenia, osteoporosis and cardiovascular events has been established [27, 28].

Hypoestrogenia is considered one of the predictors of cardiovascular risk with the formation of heart failure with the preserved systolic function of the left ventricle. Its typical clinical manifestation in postmenopausal women is diastolic dysfunction, which is caused by estrogen deficiency and the loss of its cardioprotective effect [29].

Under physiological conditions, estrogens and their receptors modulate the expression of circulating catecholamines, regulating inotropic and chronotropic effects, by signaling through β-adrenergic receptors (βAR). In conditions of hypoestrogenia, the above-mentioned factors are associated with the initiation and progression of heart failure with the preserved systolic function of the left ventricle [30, 31]. Thus, a lower incidence of the disease is observed in premenopausal women than in postmenopausal women and men in all age groups, which will be the subject of our further studies.

Unlike postmenopausal women, men retain cancellous bone integrity longer, although their bone trabeculae become thinner. During puberty, men develop larger bones than women due to periosteal appositional growth. On the other hand, in women, on the contrary, estrogens have an inhibitory effect on periosteal bone tissue formation, and a stimulating effect on endocortical bone tissue. Estrogens, by their properties, stimulate the early closure of the epiphyseal bone growth zones, which is why shorter bones are formed in women than in men. Since the amount of the formed periosteal surface of the bone tissue is greater in men than in women, endocortical resorption prevails in women [19, 26].

For women with pronounced osteopenia and osteoporosis, subject to confirmation by laboratory and other evidence-based methods, we plan to prescribe a program for the treatment of bone metabolism disorders, as well as pathogenetically related cardiometabolic complications, as the basis of preventive medicine [32, 33].

CONCLUSIONS

The results of the research prove the prevalence of osteopenic syndrome in terms of age and gender, with an emphasis on women aged 50 years and older. According to the obtained data, early detection of signs of bone mineral density disorders by screening with the help of ultrasound densitometry is appropriate and allows taking measures to prevent the progression of osteoporosis in the early stages, even before the appearance of clinical signs and its consequences. This will allow us to prevent the formation of complications, in particular, low-traumatic fractures, to develop pathogenetically based gender-oriented treatment methods to improve the quality and increase the length of life. The pathogenetic basis of postmenopausal osteoporosis is the basis for our further studies in the aspect of comorbidity.

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STUDY OF THE INFLUENCE OF LINDANE AND SODIUM BICHROMATE ON THE MYOCARDIUM

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ABSTRACT

Aim: The aim of the research is to study the immunohistochemical markers of the endothelium of blood vessels and myocardial ventricles under chronic exposure to sodium bichromate and lindane, as well as in conjunction with damaging biochemical agents contained in the blood.

Materials and Methods: The object of the experiment was outbred white mice (males). The study was carried out in 3 groups: 1st group – control, 2nd group – exposure to sodium bichromate 5 mg/kg, 3rd group – exposure to organochlorine pesticide lindane 100 mg/kg. In this experiment authors used the next methods: immunohistochemical method., biochemical research, statistical analysis.

Results: The data obtained from an experimental study show that the level of cardiomarkers in blood plasma is characterized by different changes when exposed to these two compounds. Basically, the predominance of the effect of sodium bichromate on the LDH level is noted as compared to the effect of lindane; on the CK-MB level, their effects were the same, i.e., there is an increase in their level in blood plasma.

Conclusions: Thus, long-term exposure to sodium bichromate leads to the activation of angiogenesis, destruction of the integrity of the endothelium, and this, in turn, leads to reparative changes located around in the myocardial cells.

KEY WORDS: biochemical indicators, experiment, blood vessels, cardiomarkers, immunohistochemical markers

INTRODUCTION

In modern scientific research the cellular composition of the myocardium and its angiogenesis, as well as immunohistochemical parameters that determine their morphofunctional capabilities are widely studied. Reorganization of myocardial blood vessels occurs as a result of a violation of remodeling, which is characterized by such processes as fibrosis, death of cardiomyocytes. This, in turn, is directly related to the process of apoptosis of the cellular composition of the myocardium. Cellular reorganization depends on the integrity of the blood vessel wall of the myocardium itself.

Violation of the integrity of the walls of blood vessels because of an increase in their permeability leads to the ingress of agents that affect the morphological function of cells contained in the blood, and this, in turn, leads to a change in the cellular composition or cellular apoptosis. In the studies of many scientists the functional features of the myocardium, the complexity of its structure, the features of the branching of blood vessels and changes in the microvasculature, as well as dependent changes under the influence of a number of unfavorable factors (internal and external) during their formation, changes in the endothelium in the initial periods, and local changes in the metabolism of biologically active substances have been revealed [1-3]. Various studies of the pathology associated with circulatory disorders of the structures of the heart suppose various conclusions. Current scientific projects devoted to the study of the morphology of the heart are aimed at explaining the mechanism of violation of the microvasculature of the heart wall, namely the myocardial layer, as well as preventing its occurrence.

Including the study of the features of damage to the walls of blood vessels, violation of the integrity of the endothelium, the degree of formation of foci of atherosclerosis, as well as a number of other factors leading to the remodeling of heart structures, remains relevant now [4, 5].

As it shown by results of the research investigation the violation of the permeability of the walls of blood vessels gives, first of all, a picture of violation of the integrity of the endothelium. In the structures of the peripheral circulation, the endothelium layer is the first to respond to the hemodynamic load, to the action of the agents contained in the blood. Endothelial cells together with the smooth muscle muscles of the blood vessel wall have a combined effect. Therefore, endothelial cells, reflecting indicators of morphofunctional ability, are the first to undergo changes in the action of exposure factors coming from outside, as well as those contained in the blood [6, 7].

A large place is occupied by a number of chemicals that are used in production and are among the harmful factors entering the human body from the outside. The increase in the number of people on earth has led to a shortage of food sources and foodstuffs. In this regard, in agricultural production to increase production, for long-term preservation of food, as well as in other processing

industries, the relevance of the use of pesticides remains. However, the use of these chemical compounds has adverse consequences for the body, which is reflected in scientific conclusions in the works of a number of scientists.

In countries where heavy industry (production of heavy metals) is well developed, industrial waste discharged by factories into the environment after accumulating enters the body through the air, food, exerting its opposite effect, thus forming ecologically unstable regions.

One of the largest chromium plants in the world is located in the Aktobe region of the Republic of Kazakhstan, which has led to the formation of biochemical provinces in this region. Moreover, in the neighborhood located to the Aral region with a high content of environmental pollution, in addition to the Aral zone, 5 districts of the Aktobe region were included. The aridity of the surrounding nature, the irreversible processes that have arisen as a result of the widespread use of pesticides to increase agricultural production, have led to a deterioration in living conditions of the population, as well as to morbidity. Therefore, the formation of the characteristics of diseases inherent in such ecological regions of the arising changes in the body under the influence of unfavorable environmental factors were formed in scientific research, and are also the main hypotheses in many scientific studies [8-10].

AIM

The aim of the research is to study the immunohistochemical markers of the endothelium of blood vessels and myocardial ventricles under chronic exposure to sodium bichromate and lindane, as well as in conjunction with damaging biochemical agents contained in the blood.

MATERIALS AND METHODS

The object of the experiment was outbred white mice (males) in the amount of sixty, weighing 20-30 grams (Laboratory of the Scientific and Practical Center of Marat Ospanov West-Kazakhstan Medical University Aktobe, Kazakhstan), were kept in the laboratory under a 12:12 hours light-dark schedule (light on 7 am to 7 pm) at 27±2°C and 70% humidity with free access to unlimited water and food. The study was carried out in 3 groups: 1st group – control, 2nd group – exposure to sodium bichromate ($Na_2Cr_2O_7$) 5 mg/kg, 3rd group – exposure to organochlorine pesticide Lindane ($C_6H_6Cl_6$) 100 mg/kg.

The experimental animals were injected per os for 2 months in two directions, and the control group was kept under standard vivarium conditions. To analyze the dynamics of the possible effects of toxicants, some of the mice were removed from the experiment at the end of the 1st and 2nd months of chronic exposure to chemical compounds. The animals were removed from the experiment by decapitation [11, 12].

An immunohistochemical study of the myocardium was carried out under various models of toxic effects. For immunohistochemical studies, commercial monoclonal antibodies were used: VEGF (SP28), CD 117 (RTU), p53 (Ab-5) and caveolin-1 (Cav-1) Anti-PCNA antibody [PC10] ab29 (0.01 m HEPES, ph 7.5, 0.15 m), the plasma protein was determined, which was further identified and studied under an immunofluorocent microscope.

The level of homocysteine (HC), creatin kinase MB (CK-MB) and lactate dehydrogenase (LDH) in the blood was determined by high performance liquid chromatography. This method is sensitive to the determination of total homocysteine, total glutathione (GSH + GSSG) and neuroactive amino acids (Asp, Glu, GABA) with precolumn derivation with orthophthalic aldehyde and fluorimetric determination.

For morphological analysis, a Leica DM 1000 binocular microscope + a Leica DFC290 digital camera was used; for the analysis of the results obtained, cell counting and vascular measurements were carried out using the "ImageJ soft" software. Data were analyzed using STATISTICA 10 software (Stat. Soft, Ins. USA). Using the Mann-Whitney test according to table. The reliability was determined at the level of the difference indicator at level $p<0.05$.

The design of the study was approved by the Local Ethical Commission of the Marat Ospanov West-Kazakhstan Medical University on November 19, 2021 (protocol no.9) in line with the European Convention for the Protection of Vertebrates used for experimental and other purposes (Strasbourg, 1986).

RESULTS

FEATURES OF IMMUNOHISTOCHEMICAL MARKERS UNDER THE INFLUENCE OF SODIUM BICHROMATE AND LINDANE

During the experimental study, the features in the immunohistochemical parameters that determine the functional ability of the artery endothelium and myocardial cells were revealed. According to the terms of intoxication the variability of the expression and intensity of immunohistochemical parameters was determined.

In the control group, in the endothelium, a pronounced chain of light areas in the form of caveolae is determined, in 72.3 ± 0.9% of the cells of which the expression of caveolin-1 is noted. When comparing the control group with the first month of inoculation under the influence of sodium bichromate, this indicator decreases by 2.3 times ($p<0.05$).

In the first month of the experiment, fluorescence transmission in many parts of the endothelium is not detected, in some endotheliocytes caveolin-1, and only 31.7 ± 1.1% of cells show expression. In the second month of the experiment, in the endotheliocytes of some arteries, fluorescent transillumination is not detected; only in 13.0±1.3% of cells expression of caveolin-1 is noted.

In the group primed with lindane, the expression of caveolin-1 compared with the control group is reduced, when compared with the group seeded with sodium bichromate, it is increased, where in 68.3 ± 1.2% of cells expression of caveolin-1 is noted. In the second month of seeding with lindane, discontinuous chains of clarified areas in the form of caveolae are observed in the endothelium (Fig.1).

However, in the expression of Cav-1, weakly positive cells predominate when compared to the sodium bichromate

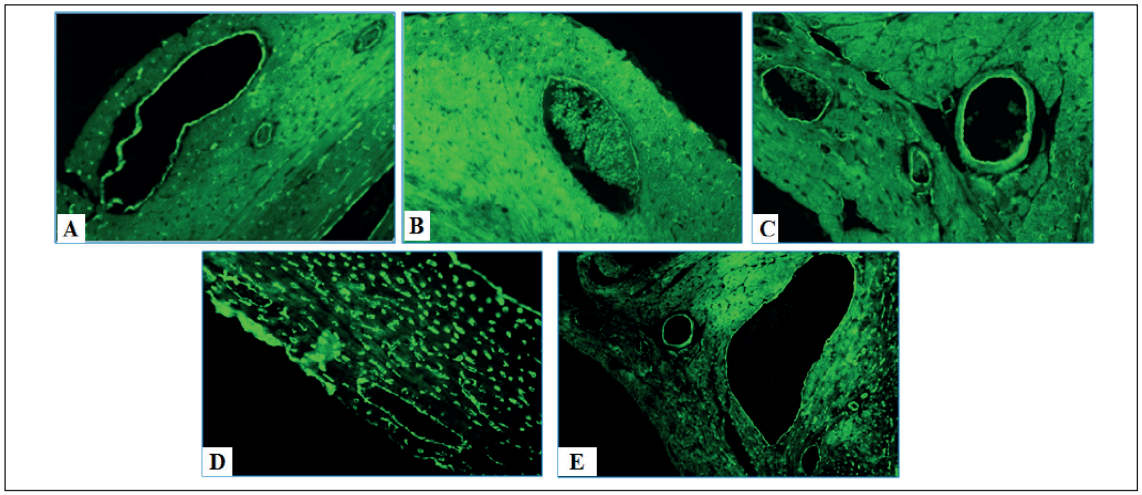


Fig 1. Expression of Cav-1 from endothelial cells. IGH. Immunofluorescence x400: (A) Expression Cav-1 in Control group (x400); (B) Expression Cav-1 the 1st month exposure to sodium bichromate (x400); (C) Expression Cav-1 the 2nd month exposure to sodium bichromate (x400); (D) Expression Cav-1 the 1st month exposure to Lindan (x400); (E) Expression Cav-1 the 2nd month exposure to Lindan (x400).

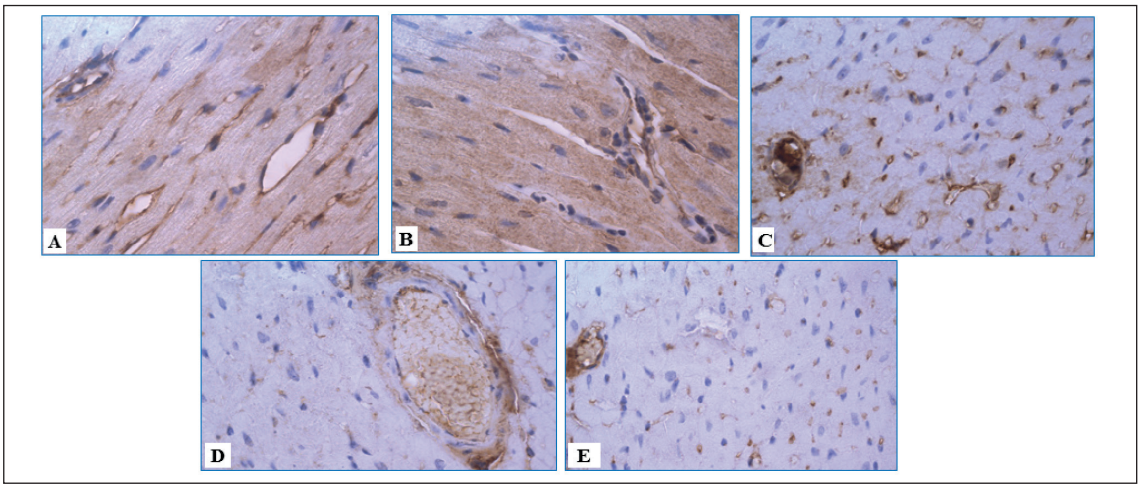


Fig 2. Immunohistochemical staining of left ventricle tissue: (A) VEGF marker expression in Control group (x800); (B) VEGF marker expression the 1st month exposure to sodium bichromate (x800); (C) VEGF marker expression the 2nd month exposure to sodium bichromate (x800); (D) VEGF marker expression the 1st month exposure to Lindan (x800); (E) VEGF marker expression the 2nd month exposure to Lindan (x800).

seed group. In 69.3 ± 0.9% of cells, expression of caveolin-1 is noted, while bCav-1 immune reactivity retains.

In the experimental group, during the first month of sodium bichromate priming, VEGF expression is determined in 29.7 ± 0.6% of the cells, when compared with the control group and the lindane priming group, the indicators are closer to each other among the groups only 1.2% of the difference is determined.

After 2 months of priming the expression of endothelial growth factor in the sodium bichromate priming group was increased by 10% compared to the control group, when this trait was reduced by only 2% in the lindane treatment group. Namely, in the lindane-primed group compared with the control group, there were no pronounced changes, and when compared with the sodium bichromate-primed group, a 12% decrease was noted ($p<0.05$) (Fig.2).

Along with this, cells expressing VEGF were detected not only in the endothelial layer, but also around small arteries, which comparatively prevailed in the group of sodium bichromate primings. When exposed to lindane, the expression of this indicator is determined around the small arteries of the myocardium located closer to the endocardium.

Changes in the structures of the endothelium of the myocardial arteries led to changes in the functional capacity of myocardial cells. The features of markers p53 and CD117 in myocardial cells characterized these changes. The markers of apoptosis in cardiomyocytes and immunohistochemical markers of mast cells were determined when exposed to sodium bichromate and lindane. Expression of immunohistochemical markers determined in the arterial endothelium directly contributes to the immunohistochemical parameters of myocardial cells. The expression of CD117 mast cells under the influence of sodium bichromate in 1 month of the experiment was 31.1 ± 1.3%, when compared with the control group, it

decreased to 15.2%, and in the 2nd month this feature was preserved, at the same time recurring changes as in the first month ($p<0.05$) (Fig.3). When exposed to lindane, CD117 positive cells had an individual characteristic. In the first month of priming, positive cells accounted for 53.9 ± 0.8%, and in the second month, 60.1 ± 1.3% (in the control group, 46.3 ± 0.9%). In this case, it was revealed that functional changes in mast cells contribute to the occurrence of apoptosis processes in cardiomyocytes located around them. In the first month of the experiment, when exposed to sodium bichromate, it was revealed that p53 positive cells were 16.9 ± 0.4% and decreased from the control group by 23.7%, and when exposed to lindane, the expression of p53 was 42.9 ± 0.6%. However, with the control group, it only increased by 2.3%.

In the second month of the experiment, in the group of sodium bichromate priming, the expression of p53 was determined in 20.3 ± 0.03% of cells, despite an increase of 4% compared to the first month of priming, a pronounced decrease in the indicator from the control group remains. When exposed to lindane, the indicator during this period of the experiment increased by 3% and from the control group by more than 5%, That is according to the expression of the p53 marker, the number of negative cells increased when exposed to sodium bichromate, and decreased when exposed to lindane compared with the control group ($p<0.05$) (Fig.4).

Thus, with prolonged exposure to sodium bichromate and lindane, changes in immunohistochemical markers of the endothelium of myocardial blood vessels contribute to the indicators responsible for the functional ability of myocardial cells located around the blood vessels, i.e., characterized by changes in the immunohistochemical parameters of mast cells and apoptosacardymyocytes.

CHANGES IN THE LEVEL OF CARDIOMARKERS IN THE BLOOD WHEN EXPOSED TO SODIUM BICHROMATE AND LINDANE

A biochemical study of blood plasma for the content of creatine kinase-MB, lactate dehydrogenase and homocystaine were characterized by different changes in their levels, which in turn proved that these chemical compounds, such as lindane and sodium bichromate, had various adverse effects. The level of homocysteine was characterized by a marked difference in the study groups when compared with the control group. The exposure to sodium bichromate in the 1st month increased by 3.8 μmol/L from the control group. And at the 2-month priming time, this indicator was 2.0 μmol/L. In the 2nd month of the experiment of seeding sodium bichromate, the level of homocysteine decreased slightly compared to the 1st month, at the same time, when compared with the control group its level is kept at high rates (Fig.5). And in the lindane primed group, in its two periods, the indicators are close to the data of the control group, and lower than in the seed group with sodium bichromate; on the contrary, the data indicate a decrease of 0.3 μmol/l ($p<0.05$).

In other words, the performance in the lindane-priming group was closer to that in the control group. Along with these changes, the level of CK-MB in the 1st month of priming with sodium bichromate increased 2.1 times, and in the 2nd month this difference was 1.7 times. This suggests that in the two periods of the experiment, the level of CK-MB in the blood plasma increased significantly in comparison with the control group. Despite the fact that its level in the 1st month of the experiment under the influence of lindane increased 2 times compared with the control group, and in the 2nd month the level of CK-MB had a pronounced decrease in comparison with the control

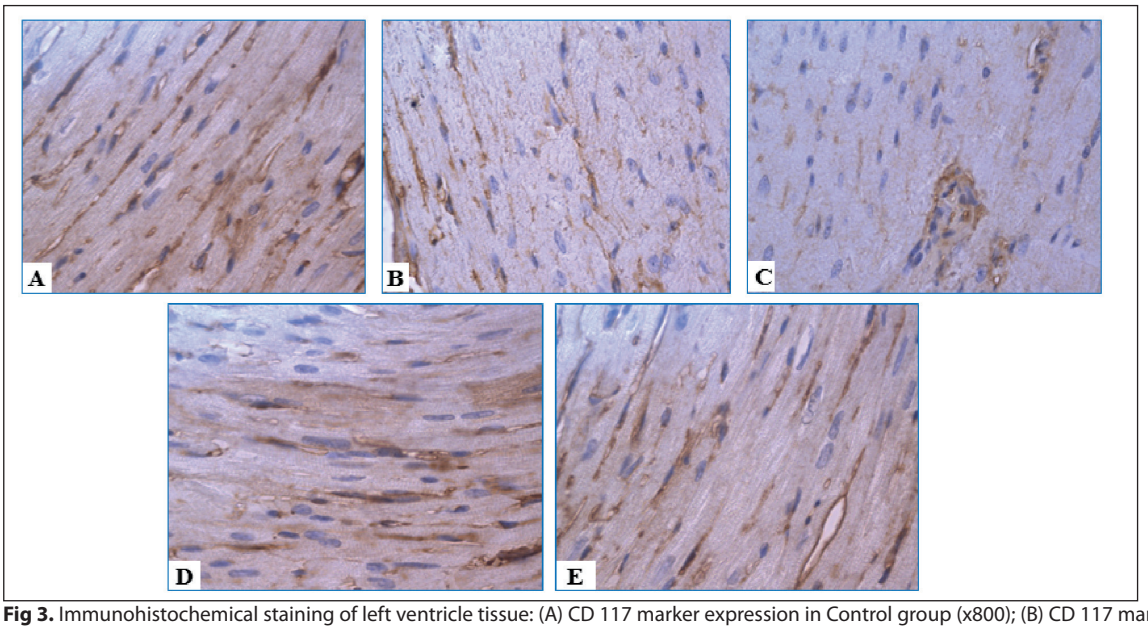


Fig 3. Immunohistochemical staining of left ventricle tissue: (A) CD 117 marker expression in Control group (x800); (B) CD 117 marker expression the 1st month exposure to sodium bichromate (x800); (C) CD 117 marker expression the 2nd month exposure to sodium bichromate (x800); (D) CD 117 marker expression the 1st month exposure to Lindan (x800); (E) CD 117 marker expression the 2nd month exposure to Lindan (x800).

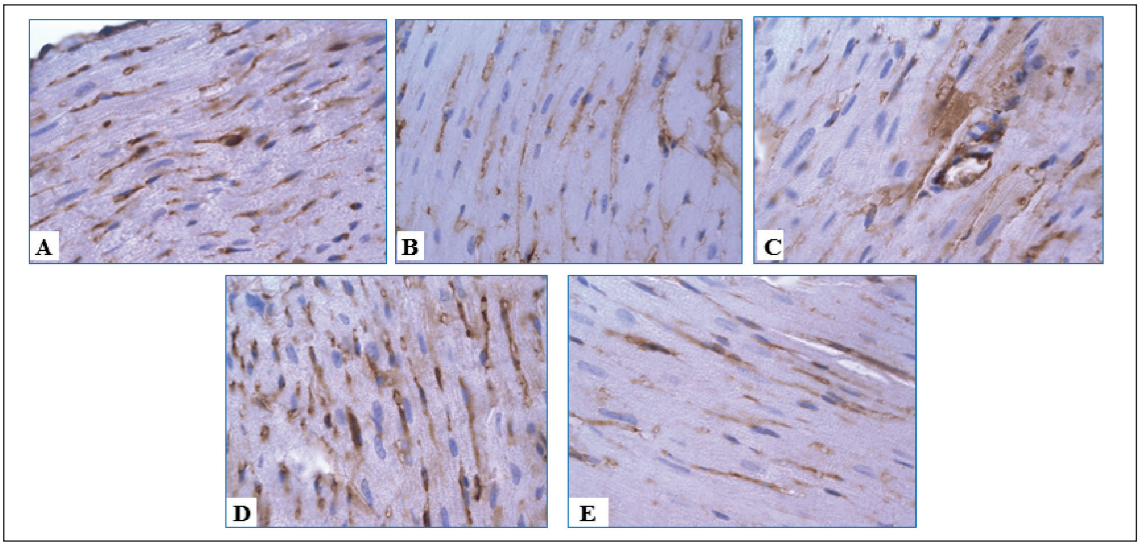
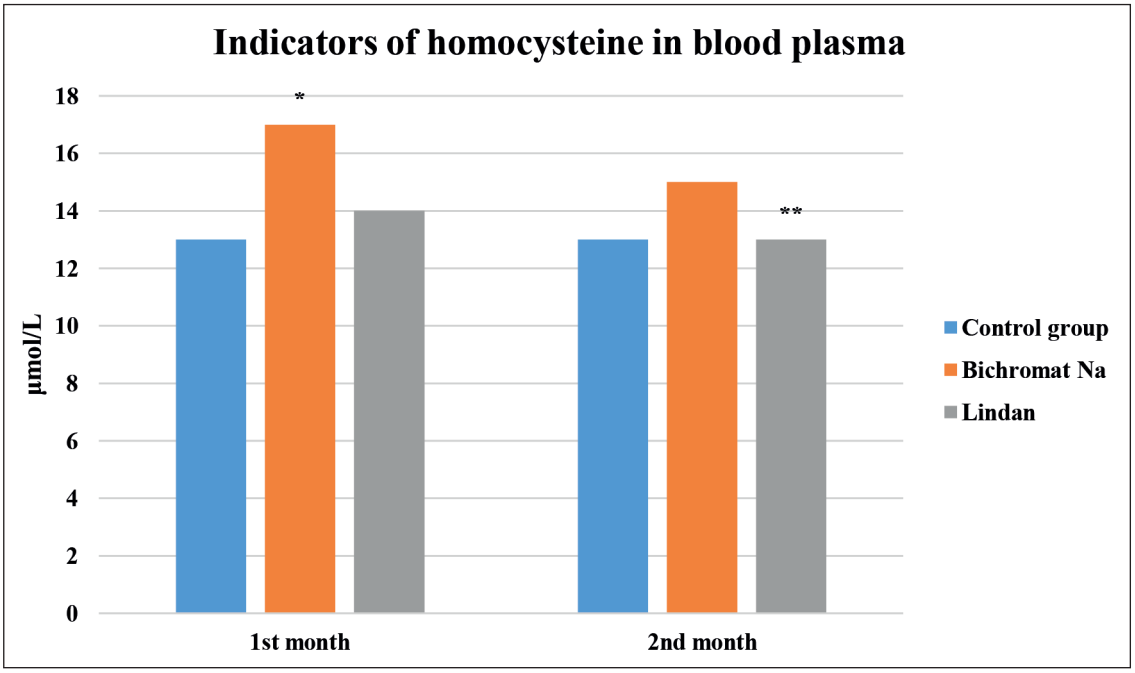


Fig 4. Immunohistochemical staining of left ventricle tissue: (A) p53 marker expression in Control group (x800); (B) p53 marker expression the 1st month exposure to sodium bichromate (x800); (C) p53 marker expression the 2nd month exposure to sodium bichromate (x800); (D) p53 marker expression the 1st month exposure to Lindan (x800); (E) p53 marker expression the 2nd month exposure to Lindan (x800).



*Note: *Obvious differences between the control group and the bichrormate sodium poisoning group p<0.05 Mann-Whitney;*
***Obvious differences between the control group and the lindane poisoning group p<0.05 Mann-Whitney.*

Fig 5. Indicators of homocysteine in blood plasma.

group and only by 189.1 U/L turned out to be higher. The level of CK-MB in blood plasma in the group of exposure to sodium bichromate was kept at high rates in two periods of the experiment. Despite an increase in its level in the 1st month of inoculation with lindane, in the 2nd month its marked decrease was noted, and its value approached the indicators of the control group. The level of LDH in blood plasma in the 1st and 2nd months of the experiment under the influence of lindane and sodium bichromate had a pronounced increase. In the 1st month of the experiment under the influence of sodium bichromate, if the LDH level increased 6.7 times compared with the control group (control -292.4±0.9 U/L, sodium bichromate – 1973.5±1.3 U/L) then in the 2nd month increased by 4.4 times, and amounted to 1291.3±1.1 U/L.

When exposed to lindane in the 1st month of the experiment, its level increased 2.7 times, and in the 2nd month it was 411.4±0.8 U/L, and it was found that the total increased 1.4 times ($p<0.05$).

Thus, the data obtained from an experimental study show that the level of cardiomarkers in blood plasma is characterized by different changes when exposed to these

two compounds. Basically, the predominance of the effect of sodium bichromate on the LDH level is noted as compared to the effect of lindane; on the CK-MB level, their effects were the same, i.e., there is an increase in their level in blood plasma.

DISCUSSION

Various chemicals, entering the body from the outside, have an effect on the cardiovascular system, thereby causing a number of changes. These changes, because of a long-term effect on the body, lead to changes in the cellular structures of the myocardium, as well as to the occurrence of reactive changes in the walls of their blood vessels through changes in the processes of repair and regeneration. Generation of myocardial blood vessels in ontogenesis, and even various adaptations to change their angioarchitectonics, has an effect on angiogenesis. Along with this, through changes in the integrity of the endothelium of blood vessels, they cause changes in the morphology of cells and tissues located around the blood vessels. It is known that local structures have a direct effect on changes in the morphofunctional ability of blood vessels located in this area [13-15]. Therefore, in this study, it is revealed that the markers p53 and CD 117, which determine apoptosis in cardiomyocytes and the functionality of mast cells, have a relationship with immunohistochemical markers of endothelial expression Cav-1 and VEGF; these immunohistochemical markers are important indicators of cardiomyocyte renewal and functional ability of mast cells. Markers indicative of endothelial growth factor and caveolae integrity take place in determining important factors such as generation of myocardial blood vessels, disruption of the integrity of the vascular wall. Along with this, the peculiarity of these immunohistochemical markers contributes to the study of damaging agents (cardiomarkers) contained in the blood plasma, and also shows the relevance and practical importance of scientific work in the study under conditions of exposure to lindane. The impact of chromium compounds on the environment is associated with the industry of large chrome processing plants in the Republic of Kazakhstan (in particular the Aktobe region), which lead to the formation of ecological provinces. Lindane, which is an organochlorine pesticide, is often used in the agricultural industry to increase yields in regions of low ecological status. In the works of many scientists, there is evidence that these compounds enter the body often through natural pathways – the digestive, respiratory and other systems provide for the development of a number of violations and changes inherent in this ecological region [16-18]. The mechanism of action and the routes of ingestion of chromium and pesticides are similar, therefore, in this study, authors conducted a comparative study of biochemical parameters (cardiomarkers) in blood plasma and immunohistochemical markers of the myocardium and its blood vessels when exposed to two compounds introduced into the stomach (experimental animals) under experimental conditions.

The expression of caveolin-1 revealed as a result of the study under the influence of sodium bichromate is marked by a significant decrease, respectively, in the dynamics of the terms of intoxication. In the data of some scientists, it is indicated that caveolin-1 is found in the isoform of caveolin 3 in the cell endothelium, they form caveolae and are directly involved in transcytosis.

Therefore, a decrease in the expression of caveolin-1, can give a picture of a violation of the integrity of the endothelium [19, 20]. These changes found in the lindane exposure group were not consistent with changes in the sodium bichromate exposure group. These changes mean that, according to the course of the experiment (first and second months), activation of vascular endothelial growth factor occurs, simultaneous flow with migration into the myocardium, disruption of the integrity of caveolin in the endothelial layer with simultaneous decreases in Cav-1 expression associated with the effect of chemical toxicants on the vascular response. Violation of the integrity of the vascular wall, increased permeability of the wall, determination of expressing VEGF cells in the myocardium, means the activity of angiogenesis. These similar changes have been identified in other scientific studies as well. According to a number of scientists, it is known that prolonged exposure to chromium compounds, as well as various metals, increasing the expression of VEGF leads to the activation of angiogenesis, by reducing the concentration of nitric oxide metabolites, endothelial dysfunction develops [21, 22].

Due to the fact that there are a large number of receptors in the endothelium, they directly perceive all stimuli; therefore, both the degree of damage and the changes occurring in them are of great importance. In this study, it was manifested by a change in the level of the damaging endothelial factor in the blood plasma – homocysteine. In the sodium bichromate group, an excess of the level of homocysteine in the form of a violation of the integrity of the endothelium occurs simultaneously with a pronounced decrease in the expression of caveolin-1 and a change in the expression of VEGF. And when exposed to lindane in its two periods, the homocysteine indices turned out to be close to those of the control group, which means that lindane does not show a general effect and has no effect on the factors contained in the blood plasma. According to the literature, this may be due to the accumulation of lindane in the tissues, i.e. because of the good solubility of lindane in fat, it accumulates in adipose tissue, and its accumulation in the blood takes the last place [23-25].

Although the effects of sodium bichromate and lindane are the same, in this experimental study, the damaging effects of sodium bichromate predominate. As shown by the results of the study, the state of mast cells expressing CD 117 promotes the processes of apoptosis in cardiomyocytes. However, the actions of both chemical compounds are not the same. Despite the fact that the expression of CD 117 when exposed to sodium bichromate in its two seeding periods significantly decreases, when exposed to lindane, on the contrary, it remains unchanged, even a slight increase is noted. The decrease in the markers of apoptosis p53 of cardiomyocytes and the expression marker of CD 117 of mast cells, revealed in the experiment, gives a picture of a violation of the reparative capacity of cells, namely, the destruction of cardiomyocytes in a

natural way is impaired, this may be a picture of a change in the direction of a pathological process. A number of scientists in their studies have revealed that intracellular inflammation of cardiomyocytes, their contraction and energy capacity, can lead to impaired apoptosis.

After the entry of chromium (VI) into the body at the cellular level, a connection is established between the protein and DNA, and it has been proven that changes occurring in them lead to mutation. Along with this, a number of scientists in their studies have proved that in the microvasculature they are enhanced in the form of a vascular reaction [17, 26, 27]. Immunohistochemical changes in myocardial cells occur simultaneously with changes in a number of blood plasma enzymes involved in metabolism, proceeding simultaneously with a decrease in the functional capacity of myocardial cells, lead to a violation of their morphological structure [28-30].

CONCLUSIONS

The level of creatine kinase-MB fraction upon exposure to sodium bichromate in the first and second months of inoculation is significantly increased. And when exposed to lindane, its level in the first month is increased, when in the second month its level is the same as in the control group. The same changes were found in the LDH level. Despite the fact that the level of LDH is significantly increased in two periods of inoculation when exposed to sodium dichromate, then when exposed to lindane, the level of its value in two periods is characterized by the same indicators. This demonstrates that sodium bichromate causes significant changes in the level of cardiomarkers contained in the blood, in comparison with lindane, and also corresponds to changes in immunohistochemical parameters.

An increase in the level of myocardial damaging markers in blood plasma, the effect on the expression of immunohistochemical markers characterizing the functional ability of myocardial cells, demonstrates an effect on compensatory-reparative processes. According to the literature data, among chromium compounds, long-term action causes hematological changes, and leads to changes in apoptosacardiomyocytes in the structure of the heart.

At the same time, it is known that a number of pesticides have a cardiotoxic effect and these chemical compounds lead to such processes as apoptosis, degeneration, vacuolization, excessive intake of organophosphate pesticides into the body leads to an increase in the level of myocardial damaging markers in blood plasma, and these changes, respectively, were revealed in this research.

Thus, long-term exposure to sodium bichromate leads to the activation of angiogenesis, destruction of the integrity of the endothelium, and this, in turn, leads to reparative changes located around in the myocardial cells. These changes showed that the level of cardiomarkers contained in the blood plasma, according to the timing of the inoculation, proceeds in a wave-like change. Despite the fact that when primed with lindane, similar changes were revealed, it was still significantly weaker compared to the effect of sodium bichromate.

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THE RELATIONSHIP BETWEEN WORK CHARACTERISTICS AND SUBJECTIVE WELL-BEING OF EDUCATIONAL ORGANIZATION MANAGERS AND EMPLOYEES

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ABSTRACT

Aim: To empirically investigate the relationship between work characteristics and subjective well-being of managers and employees in educational organizations.

Materials and Methods: Analysis of work characteristics was done using The Work Design Questionnaire (WDQ). The modified BBC Subjective Well-being scale (BBC-SWB) was used to analyze educational staff's well-being. The research data were processed using mathematical statistics: descriptive statistics (mean, std. deviation), Pearson correlation, and independent samples t-test. 345 respondents from the Central region of Ukraine participated in the survey.

Results: Although educational staff had a generally positive assessment of work characteristics, there were certain disproportions in their assessments of individual work characteristics: work autonomy characteristics were rated lower than task characteristics, while social characteristics and work context characteristics were rated lower than knowledge characteristics. In addition, educational organization managers rated the vast majority of work characteristics higher than employees, which suggests a more favorable organizational environment for managers than employees. There were three work characteristics groups in the context of their associations with staff's subjective well-being: 1) work characteristics that had a positive statically significant relationship with both managers and employees' subjective well-being indicators; 2) work characteristics that had positive and negative statistically significant relationships with employees' subjective well-being indicators; 3) work characteristics that had positive and negative statistically significant relationships with managers' subjective well-being indicators.

Conclusions: The obtained findings may be helpful for managers and employees of educational organizations in promoting staff's subjective well-being and increasing educational organizations' effectiveness.

KEY WORDS: work characteristics, subjective well-being, education managers, education employees

INTRODUCTION

The transformations that have been taking place in Ukraine are accompanied by intense activities of organizations, particularly educational ones because of their significant role in innovations that are aimed at bringing the Ukrainian system of education in compliance with the European standards. However, the innovations in education are often not prepared, both in terms of management and financially. Besides, they are taking place in the conditions of the ongoing economic crisis and the military conflict in Ukraine, as well as the global pandemic, which can cause mental health problems and threaten well-being of the staff of educational organizations [1-3]. Educational staff's endangered well-being, in its turn, can negatively affect the work of educational institutions because it correlates with teaching effectiveness and educational institution's overall performance [4-9].

Today, there are many approaches to determining well-being. In our research, we follow the concept of well-being proposed by P. Kinderman, M. Schwannauer, and E. Pontin [10-12] who, based on the research of J. Beddington et al. [13], define subjective well-being as a state "in which a person is able to develop their potential, work productively and creatively, build strong and positive relationships with others and contribute to their community" [10]. The researchers emphasize the need to distinguish subjective well-being from objective external factors that determine or influence well-being, e.g. material (e.g., housing) and/or financial (e.g., income) factors. It is important, in our opinion, that the model of subjective well-being is based on an integrative approach and includes physical, psychological, and social components [10-12].

Analysis of the problem of staff's subjective well-being has found that besides subjective factors [14-16] work and

organization's characteristics are also reliable predictors of staff's well-being [16-21].

Teachers' subjective well-being has also been the subject of many studies [15, 22, 23]. It was found that teachers' coping strategies affected their psychological well-being [24-26]. R. Acton and P. Glasgow [4] define teachers' well-being as "an individual sense of personal professional fulfillment, satisfaction, purposefulness and happiness created in cooperating with colleagues and students" [4].

Probably, the most comprehensive description of work characteristics was made by P.F. Morgeson and S.E. Humphrey [27] who offered a three-component structure of work design. The first component includes motivational characteristics that reflect overall work complexity. The work will be more motivating and satisfying if it has high motivational characteristics, which include work task characteristics and knowledge characteristics. The second component is made up of the social characteristics of work, which describe the work in terms of social environment. The third component includes contextual characteristics of work that describe its physical and environmental contexts [27].

In line with the proposed model, the researchers developed the Work Design Questionnaire, which was translated into a number of languages [28-30] and adapted for use in many countries.

The use of WDQ in other studies allowed finding statistically significant relationships with such stress indicators as job satisfaction [29] and work engagement [31]. For example, in the study by Z.R. Kahaki, et al. [29] stress and job satisfaction variables showed a negative and positive relationship with the WDQ parameters, respectively.

However, the relationships between work characteristics and educational organization managers and employees' subjective well-being have not been analyzed in the relevant psychological literature.

AIM

To empirically investigate the relationship between work characteristics and subjective well-being of managers and employees in educational organizations; to analyze the differences in managers and employees' assessments of work that relate to its task characteristics, knowledge characteristics, social characteristics, and general context.

MATERIALS AND METHODS

SETTING

The sample was made up of 345 respondents: 101 secondary school managers (principals, vice-principals, heads of departments) and 244 secondary school teachers. The sample included 88% females and 12% males aged 23 through 67 years (M = 41.97, SD = 10.79) from the Central region of Ukraine.

DATA COLLECTION TOOLS

The Work Design Questionnaire (WDQ) (Morgeson& Humphrey, 2006) was used to analyze 21 job characteristics, which were divided into task, knowledge, social, and contextual groups (Task characteristics, Knowledge characteristics, Social characteristics, Contextual characteristics).

The modified BBC Subjective Well-being scale (BBC-SWB) (Pontin, Schwannauer, Tai, & Kinderman, 2013) was used to analyze teacher well-being on the scale of *well-being* (overall) and three sub-scales (Psychological well-being, Physical health and well-being, Relationships). The Modified BBC-SWB has 24 statements and uses the 5-points Likert scale.

DATA ANALYSIS

The research data were processed using mathematical statistics (SPSS 22.0 for Windows): descriptive statistics (mean, std. deviation), Pearson correlation, and independent samples t-test. The data were collected in 2021 using Google Form.

RESULTS

EDUCATIONAL ORGANIZATION STAFF'S ASSESSMENT OF WORK CHARACTERISTICS AND ASSESSMENT OF DIFFERENCES BETWEEN MANAGERS AND EMPLOYEES

Table 1 presents the data that relate to the assessment of job characteristics by the staff of educational organizations.

Regarding the *task characteristics* group, which consisted of task autonomy characteristics and the characteristics that relate directly to tasks, the following results were obtained. The characteristics that related directly to tasks had mainly high or almost high scores: task variety (M = 4.10, SD = 0.72), task significance (M = 3.96, SD = 0.67), task identity (M = 3.88, SD = 0.73), and feedback from job (M = 3.86, SD = 0.69). At the same time, the characteristics related to task autonomy were at an average level: work methods autonomy (M = 3.79, SD = 0.74), work scheduling autonomy (M = 3.73, SD = 0.73), and decision-making autonomy (M = 3.63, SD = 0.78). That is, the characteristics of work autonomy were assessed by the staff of educational organizations lower than the characteristics that related directly to the tasks themselves.

The majority of the *knowledge characteristics* group were highly assessed by the educational organization staff: skill variety (M = 4.22, SD = 0.72), information processing (M = 4.18, SD = 0.78), and problem solving (M = 3.99, SD = 0.68), while specialization (M = 3.42, SD = 0.74) and job complexity (M = 3.12, SD = 0.88) had average scores.

In the social characteristics group, only social support had high scores (M = 4.08, SD = 0.66), while the rest of the characteristics had average scores: feedback from others (M = 3.68, SD = 0.78), interaction outside organization (M = 3.58, SD = 0.86), initiated interdependence (M = 3.45, SD = 0.85), and received interdependence (M = 3.22, SD = 0.89). Thus, it can be concluded that, in general, social characteristics of work are less represented in educational organizations compared to task characteristics and knowledge characteristics.

Although most of the *work context* group of characteristics were at an average level (ergonomics (M = 3.34, SD = 0.70), work conditions (M = 3.27, SD = 0.72), and equipment use (M = 3.14, SD = 0.82)), this group's one characteristic (physical demands) received low scores (M = 2.73, SD = 1.05). It's noteworthy that none of the work context characteristics had high scores.

In general, it can be concluded that task characteristics and knowledge characteristics were assessed by the staff of

Table 1. Descriptive statistics on whole sample and comparison of averages between educational organization managers and employees by work characteristics

Work characteristics	Whole sample (n=345)		Employees (n=244)		Managers (n=101)		t	p
	M	SD	M	SD	M	SD		
Task characteristics								
Work Scheduling Autonomy	3.73	0.73	3.65	0.74	3.92	0.65	-3.23	0.001
Decision-Making Autonomy	3.63	0.78	3.62	0.77	3.66	0.82	-0.41	0.685
Work Methods Autonomy	3.79	0.74	3.77	0.75	3.84	0.71	-0.88	0.378
Task Variety	4.10	0.72	4.01	0.77	4.33	0.54	-3.79	0.000
Task Significance	3.96	0.67	3.89	0.69	4.12	0.59	-2.99	0.003
Task Identity	3.88	0.73	3.86	0.74	3.93	0.70	0.80	0.425
Feedback From Job	3.86	0.69	3.83	0.69	3.93	0.70	-1.31	0.190
Knowledge Characteristics								
Job Complexity	3.12	0.88	3.03	0.88	3.32	0.87	-2.72	0.007
Information Processing	4.18	0.78	4.14	0.79	4.27	0.76	-1.44	0.150
Problem Solving	3.99	0.68	3.95	0.71	4.09	0.58	-1.73	0.085
Skill Variety	4.22	0.72	4.15	0.74	4.39	0.61	-2.87	0.004
Specialization	3.42	0.74	3.45	0.76	3.34	0.69	1.15	0.252
Social Characteristics								
Social Support	4.08	0.66	4.01	0.71	4.27	0.45	-4.12	0.000
Initiated Interdependence	3.45	0.85	3.36	0.86	3.66	0.79	-3.05	0.002
Received Interdependence	3.22	0.89	3.20	0.80	3.26	1.09	-0.51	0.613
Interaction Outside Organization	3.58	0.86	3.55	0.87	3.66	0.84	-1.04	0.301
Feedback From Others	3.68	0.78	3.59	0.79	3.90	0.71	-3.44	0.001
Work Context								
Ergonomics	3.34	0.70	3.32	0.70	3.40	0.71	-0.99	0.319
Physical Demands	2.73	1.05	2.89	1.02	2.34	1.03	4.55	0.000
Work Conditions	3.27	0.72	3.30	0.76	3.22	0.60	0.91	0.362
Equipment Use	3.14	0.82	3.25	0.82	2.88	0.77	3.82	0.000

educational organizations higher than the characteristics of work that related to autonomy, social aspect and work context.

Statically significant differences were found between educational organization managers and employees' assessments of work characteristics (Table 2). It should be noted that these differences were observed in each group of work characteristics.

There were found statistically significant differences between managers and employees' assessments of the following *task characteristics*: work scheduling autonomy ($t = -3.23$, $p < 0.01$), task variety ($t = -3.79$, $p < 0.001$), and task significance ($t = -2.99$, $p < 0.01$). Managers assessed these work characteristics higher than employees.

Also, there were statistically significant differences between managers and employees' assessments of the following *knowledge characteristics:* job complexity ($t = -2.72$, $p < 0.01$), problem solving ($t = -1.73$, $p < 0.1$), skill variety ($t = -2.87$, $p < 0.01$). Managers assessed these work characteristics higher than employees.

Higher statistically significant scores were given by managers rather than employees to the following *social characteristics*: social support ($t = -4.12$, $p < 0.001$), initiated interdependence ($t = -3.05$, $p < 0.01$), and feedback from others ($t = -3.44$, $p < 0.01$).

The following *work context* characteristics, on the contrary, received higher scores from employees rather than managers: physical demands ($t = 4.55$, $p < 0.001$) and equipment use ($t = 3.82$, $p < 0.001$).

In general, the study revealed differences in the assessments of work characteristics by educational organizations managers and employees, with managers usually giving higher scores to working characteristics than employees.

RELATIONSHIPS BETWEEN WORK CHARACTERISTICS AND EDUCATIONAL STAFF'S SUBJECTIVE WELL-BEING INDICATORS

Table 2 shows the data on the relationship between educational organization staff's assessment of work characteristics and of subjective well-being indicators. As can be seen from the table, there was a large number of statistically significant relationships between the two groups of work indicators, which were manifested differently

in different groups of job characteristics and subjective well-being components.

As for the *task characteristics* of work, positive statistically significant relationships (p<0.001) were found between *work methods autonomy* and *task variety* and all subjective well-being indicators (psychological well-being, physical health and well-being, relationships, total well-being), both for managers and employees.

Somewhat weaker but still positive statistically significant were relationships (p<0.05; p<0.01; p<0.001) between *work scheduling autonomy* and *task significance* and all subjective well-being indicators, both for managers and employees (except for one indicator for employees and managers).

At the same time, there were differences in the relationships between these work characteristics and subjective well-being: decision-making autonomy had positive statistically significant relationship (p<0.001) with all indicators of managers' subjective well-being. However, no statistically significant relationships were found between decision-making autonomy and any of the of subjective well-being indicators in education employees.

Task identity and feedback from job, on the contrary, had positive statistically significant relationships (p<0.001) with all employees' subjective well-being indicators, while managers didn't have this relationship.

Thus, a number of task characteristics are equally significant for the subjective well-being of both employees and managers, but other job characteristics have different meanings for the subjective well-being of employees and managers.

With regard to the second group of work characteristics, *knowledge characteristics*, *job complexity* had positive

Table 2. The relationship between work characteristics and educational organization managers and employees' subjective well-being indicators

Work characteristics	Psychological well-being		Physical health and well-being		Relationships		Total well-being	
	Employees (n=244)	Managers (n=101)	Employees (n=244)	Managers (n=101)	Employees (n=244)	Managers (n=101)	Employees (n=244)	Managers (n=101)
Task characteristics								
Work Scheduling Autonomy	0.267***	0.301**	0.089	0.289**	0.136*	0.242*	0.206***	0.413***
Decision-Making Autonomy	0.124	0.329***	0.039	0.527***	0.079	0.361***	0.099	0.403***
Work Methods Autonomy	0.205***	0.249*	0.173**	0.199*	0.174**	0.329***	0.217***	0.291**
Task Variety	0.244***	0.395***	0.199**	0.376***	0.136*	0.320***	0.234***	0.462***
Task Significance	0.230***	0.322***	0.198**	0.033	0.156*	0.293**	0.233***	0.224*
Task Identity	0.315***	0.189	0.287***	0.052	0.225***	0.139	0.327***	0.123
Feedback From Job	0.318***	0.336***	0.239***	0.205	0.236***	0.129	0.316***	0.147
Knowledge Characteristics								
Job Complexity	-0.131*	0.243*	-0.266***	0.216*	-0.162**	0.145	-0.195**	0.246*
Information Processing	0.208***	0.268**	0.106	0.139	0.087	0.264**	0.171**	0.238*
Problem Solving	0.209***	0.157	0.204***	-0.114	0.171**	0.133	0.227***	0.211*
Skill Variety	0.172**	0.006	0.054	-0.017	0.135*	0.142	0.147*	0.049
Specialization	0.175**	-0.064	0.173**	-0.064	0.261***	-0.078	0.222***	-0.188
Social Characteristics								
Social Support	0.228***	0.400***	0.250***	0.342***	0.277***	0.538***	0.282***	0.574***
Initiated Interdependence	0.068	0.107	0.044	-0.020	0.229***	0.118	0.112	0.088
Received Interdependence	0.024	0.262**	0.124	0.187	0.098	0.190	0.083	0.067
Interaction Outside Organization	0.276***	-0.031	0.341***	-0.180	0.239***	-0.065	0.329***	-0.075
Feedback From Others	0.177**	0.149	0.233***	0.002	0.063	0.233*	0.191**	0.159
Work Context								
Ergonomics	0.220**	-0.044	0.149*	-0.014	0.258***	-0.040	0.181**	-0.069
Physical Demands	0.265***	-0.279**	0.420***	-0.175	0.290***	-0.098	0.366***	-0.008
Work Conditions	0.227***	0.235*	0.375***	0.323***	0.197**	0.133	0.304***	0.355***
Equipment Use	0.093	-0.268**	0.150*	-0.082	0.144*	-0.030	0.140*	-0.313**

** p<0.05;** p<0.01; *** p<0.001*

statistically significant relationships with all managers' subjective well-being indicators and negative statistically significant relationships with all employees' subjective well-being indicators ($p<0.05$; $p<0.01$; $p<0.001$): an increase in job complexity reduced all employees' subjective well-being indicators but increased all managers' subjective well-being indicators.

Statistically significant differences ($p<0.05$; $p<0.01$; $p<0.001$) between managers and employees were also found regarding such job characteristics as *problem solving*, *skill variety*, and *specialization:* an increase in these job characteristics increased almost all employees' subjective well-being indicators, but had no statistically significant effect on most of managers' subjective well-being indicators.

It is noteworthy that such work characteristics as *information processing* and *skill variety* did not have statistically significant relationships with managers and employees' *physical health and well-being* although they had statistically significant relationships with other subjective well-being indicators.

It can be concluded that managers and employees differ in almost all *knowledge characteristics'* relationships with their subjective well-being.

Social characteristics of work also were found to have some relationships with the respondents' well-being. Unlike *social support* that had statistically significant relationships ($p<0.05$; $p<0.01$; $p<0.001$) with all indicators of subjective well-being of both managers and employees, *interaction outside the organization* and *feedback from others* had statistically significant relationships ($p<0.01$; $p<0.001$) with the majority of employees' subjective well-being indicators but had no such relationships with managers' subjective well-being indicators. In other words, *interaction outside the organization* and *feedback from others* as work characteristics affected employees' subjective well-being and did not affect managers' subjective well-being.

Such work characteristics as *initiated interdependence* and *received interdependence,* in fact, did not have statistically significant relationships with employees' subjective well-being, but had such relationships with some of the managers' subjective well-being indicators. Thus, *initiated interdependence* statistically significantly associated with managers' *relationships* ($p<0.001$), while *received interdependence* with managers' *psychological well-being* ($p<0.01$).

Thus, it can be concluded that the social and task characteristics of work have different statistically significant relationships with educational staff' subjective well-being: some work characteristics are equally significant for the subjective well-being of both employees and managers, but other work characteristics are related to the subjective well-being of either managers or employees.

As to the relationships between educational staff's subjective well-being and the work characteristics that belonged to *work context* group, the following results were obtained. As was the case with other work characteristics groups, some work context characteristics had a positive effect on the subjective well-being of both managers and employees, while others had positive effects on either managers or employees' subjective well-being. *Work conditions*, for example, had positive statistically significant relationships ($p<0.05$; $p<0.01$; $p<0.001$) with the vast majority of managers and employees' subjective well-being indicators. In other words, improvement of working conditions had a positive effect on most subjective well-being indicators of both managers and employees.

Another work context characteristic, *physical demands*, had positive statistically significant relationships ($p<0.001$) with all of the employees' subjective well-being indicators and a statistically significant negative relationship ($p<0.01$) with managers' psychological well-being. That is, an increase in physical demands increased employees' subjective well-being but decreased managers' psychological well-being.

The similar results were obtained on *equipment use* work context characteristic: the higher scores on this characteristic, the better the most of employees' subjective well-being indicators ($p<0.05$), but the lower managers' psychological well-being indicators ($p<0.01$) and total well-being ($p<0.01$).

This was also the case with such a work context characteristic as *ergonomics*: high levels of ergonomics associated with better employees' subjective well-being ($p<0.05$; $p<0.01$; $p<0.001$), but did not associate with managers' subjective well-being.

Thus, *work context* characteristics are differently related to educational staff's subjective well-being. Some characteristics are equally significant for the subjective well-being of both employees and managers, but others have a positive effect on employees' subjective well-being and a negative effect on individual indicators of managers' subjective well-being, such as *psychological well-being* and *physical health and well-being*. It is noteworthy that work context characteristics, compared to other work characteristics groups, had the greatest number of negative associations with managers' subjective well-being.

DISCUSSION

The article presents the results of a study on the relationship between educational organization managers and employees' assessments of work characteristics and their subjective well-being.

It was found that, in general, educational organization staff rated most of work characteristics as high or average. However, the work characteristics that related to work tasks (task variety, task significance, tasks identity, feedback from work) were rated higher than the characteristics of work that related to work autonomy (work scheduling autonomy, decision-making autonomy, and work methods autonomy). Also, many work components related to knowledge characteristics (information processing, problem solving, and skills variety) had higher scores compared to work social characteristics and work context characteristics. This research findings completely coincide with the results obtained by the authors of the Work Design Questionnaire received in organizations of various specializations including education P.F. Morgeson, S.E. Humphrey [27]. Therefore, educational organizations should focus on promoting staff autonomy and social interaction, as well as on creating favorable work conditions.

There were differences in educational organization managers' and employees' assessments of work characteristics: unlike employees, managers rated higher the vast majority of work characteristics that belonged to task characteristics (work scheduling autonomy, task variety, and task significance), knowledge characteristics (work complexity, problem solving, and skills variety) and social characteristics (social support, initiated interdependence, and feedback from work). However, educational organization employees rated the work context characteristics (physical demands and equipment use) higher than managers. This suggests that the managers of educational organizations are in a better organizational and working environment compared to employees, which can be explained, on the one hand, by the higher managers' status and their greater work opportunities, and on the other hand, by their management styles (authoritarian, liberal, democratic) and use of traditional or modern methods of employees' work organization (team work, project method), which can have different effects on employees' activity, initiative, and autonomy. A number of foreign studies have emphasized the importance of these employees' characteristics, in particular, in the conditions of distance learning, the intensity of which has increased dramatically during the Covid-19 pandemic [32-33].

Our investigation found statistically significant relationships between work characteristics and educational organization staff's subjective well-being.

It was established that a number of work characteristics had positive statically significant relationships with all or the majority of subjective well-being indicators, both for *managers and employees*. Those work characteristics belonged to the following groups: a) task characteristics (work methods autonomy, task variety, work scheduling autonomy, and tasks significance; b) social characteristics (social support); c) work context (working conditions). This means that increasing these work characteristics increases the subjective well-being of both managers and employees.

At the same time, a number of work characteristics had both positive and negative statistically significant relationships with all or the majority of *only employees'* subjective well-being indicators: task characteristics (task identity and feedback from work), knowledge characteristics (problem solving and skills variety), social characteristics (interaction outside organization and feedback from others), work context characteristics (physical demands and ergonomics) had positive relationships with employees' subjective well-being, while work complexity, which belongs to the knowledge characteristics, had a negative effect on employees subjective well-being.

It was also found that a number of work characteristics had both positive and negative statistically significant relationships with all or the majority of *only managers'* subjective well-being indicators: task characteristics (decision-making autonomy), knowledge characteristics (work complexity), and social characteristics (initiated interdependence and received interdependence) had positive effects on managers' subjective well-being indicators, while physical demands, and equipment use, which belong to work context characteristics, had negative effects on managers' subjective well-being indicators.

The obtained data are in line with the findings of some foreign studies. Thus, R. Collie and others [5, 10] in their study of teachers, found that those who had more positive relationships with students and colleagues tended to have higher levels of well-being. Therefore, the efforts aimed at developing and maintaining strong social ties are crucial, especially in times of a pandemic, when people are likely to have fewer social interactions than usual [5].

H. Yin, S. Huang, and W. Wang [9] proved the importance of a reliable school environment for meeting teachers' needs and protecting their subjective well-being. The researchers emphasized the importance of school administrators' focus on the development of trusting relationships between colleagues and the promotion of the atmosphere of authenticity in schools [9].

In our opinion, a significant advantage of our research is a holistic view of employees' work characteristics in the context of their subjective well-being.

Despite the sample's large size, it cannot be considered representative of the entire population of Ukraine, since it consisted of the inhabitants only from the Central region of Ukraine, which is more socially and economically stable than other regions, and included more females than males.

The survey was not previously validated as it was created specifically for this study. However, due to the descriptive and exploratory nature of the study, the survey provided a good link between the research question and a survey format that was not burdensome to the respondents.

CONCLUSIONS

It was found that work characteristics were assessed mainly positively by educational organization staff. However, there was a certain disproportion in the assessment of various work characteristics: work autonomy was rated lower compared to task characteristics; social characteristics and work context characteristics were rated lower compared to knowledge characteristics. Thus, it seems reasonable for the educational organization management to promote staff's work autonomy and social interaction, as well as organizational, technological, and ergonomic working conditions.

Educational organization managers rated the vast majority of work characteristics higher than employees, which suggests that managers work in a better organizational environment than employees and employees' working conditions should also be improved.

Three groups of work characteristics were distinguished in terms of their relationships with the educational staff's subjective well-being: 1) a group of characteristics that had positive statically significant relationship with all (or the majority of) subjective well-being indicators of both managers and employees; 2) a group of characteristics that had both positive and negative statistically significant relationships with all (or the majority of) subjective well-being

indicators of only employees; 3) a group of characteristics that had both positive and negative statistically significant relationships with all (or the majority of) subjective well-being indicators of only managers. This suggests that educational organization management should promote the work characteristics that contribute to staff's subjective well-being as well as those that are critical to different staff categories' (managers and employees) subjective well-being.

The research findings on the relationships between work characteristics and educational organization staffs' subjective well-being can be helpful for improvement of educational organization management focusing both on work results and on meeting staff's needs.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest.

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ER22/23EK AND TTH111I POLYMORPHIC VARIANTS IN THE GLUCOCORTICOID RECEPTOR GENE IN PATIENTS WITH BRONCHIAL ASTHMA

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ABSTRACT

Aim: The objective of the study was to evaluate the frequency of the ER22/23EK and Tth111I polymorphic variants in the glucocorticoid receptor (GR) gene in patients with BA and to assess the risk of BA development with regard to these polymorphisms.

Materials and Methods: We examined 553 BA patients and 95 apparently healthy individuals. BA was diagnosed according to the 2016 GINA recommendations and its later versions. The study was approved by the Bioethics Committee of the Medical Institute of Sumy State University. The ER22/23EK (rs 6189/6190) and Tth111I (rs10052957) polymorphisms in the GR gene were determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS–17 program.

Results: The obtained distribution of genotypes for the ER22/23EK and Tth111I polymorphisms in the GR gene corresponded to the Hardy-Weinberg expectations ($p > 0.05$). We revealed no significant difference in the distribution of alleles and genotypes for the ER22/23EK polymorphism in the GR gene in patients with asthma and apparently healthy individuals ($\chi^2 = 4.14$; $p = 0.126$); apart from that, we found no statistically significant association with BA risk in any model of inheritance. A statistically significant difference was observed in the distribution of genotypes for the Tth111I polymorphism in the GR gene in patients with asthma and apparently healthy individuals ($\chi^2 = 6.278$; $p = 0.043$). BA risk was 2.69 times higher in the carriers of TT genotype for the Tth111I polymorphism in the GR gene vs. major allele carriers. No gender-specific difference was observed in the distribution of genotypes and alleles for the ER22/23EK and Tth111I polymorphisms in the GR gene.

Conclusions: We found no gender-specific difference in the distribution of alleles and genotypes for the ER22/23EK and Tth111I polymorphisms in the GR gene; no difference in the distribution of alleles and genotypes for the ER22/23EK polymorphism in the GR gene in patients with asthma and apparently healthy individuals; and no statistically significant association with BA risk. A statistically significant difference was observed in the distribution of genotypes for the Tth111I polymorphism in the GR gene in patients with asthma and apparently healthy individuals; also, BA risk was 2.69 times higher in the minor allele homozygous patients vs. major allele carriers.

KEY WORDS: asthma, glucocorticoid receptor gene, ER22/23EK, Tth111I gene polymorphisms

INTRODUCTION

Bronchial asthma (BA) is a polygenic disease, the pathogenesis of which involves many interrelated structural genes, gene modifiers, etc. The study of the role of ER22/23EK and Tth111I polymorphic variants in the glucocorticoid receptor (GR) gene was due to the fact that the involvement of some of its polymorphic variants in the development of asthma [1 - 5] and metabolic syndrome was of scientific interest [6 - 8]. Among 2,571 mutations in the GR gene, only the following 5 single-nucleotide substitutions are observed in the Caucasian population with a frequency of > 1%: rs6190 (Arg23Lys), rs6195 (N363S), rs41423247 (BclI, C/G), and rs10052957 (-3807 C/T or Tth111I) [9]. These SNPs are associated with GR malfunctions , impaired sensitivity to glucocorticoids [10,11], and obesity and BA risk [2, 5, 9]. We chose the Tth111I and ER22/23EK polymorphisms in the GR gene to be studied due to their interrelation because ER22/23EK polymorphism was always associated with the T-allele of the Tth111I polymorphism, while this haplotype, in turn, was associated with relative resistance to glucocorticoids [10].

AIM

The objective of the study was to evaluate the frequency of the ER22/23EK and Tth111I polymorphic variants in the GR gene in patients with BA and to assess the risk of BA development.

MATERIALS AND METHODS

We examined 553 BA patients (experimental group) and 95 apparently healthy individuals (control group). BA was diagnosed according to the 2016 GINA recommendations and its later versions.

The study was approved by the Bioethics Committee of the Medical Institute of Sumy State University. The ER22/23EK

(rs 6189/6190) and Tth111I (rs10052957) polymorphisms in the GR gene were determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS–17 program. We used Pearson's chi-squared test; the P-value of <0.05 was considered statistically significant. Binary logistic regression was used for risk assessment; the analysis-of-variance method and F-test were also used in the study.

RESULTS

In order to find a possible association of Tth111I and ER22/23EK polymorphisms in the GR gene with BA risk, we performed statistical analysis. The obtained distribution of genotypes for the ER22/23EK and Tth111I polymorphisms in the GR gene corresponded to the Hardy-Weinberg expectations (p > 0.05). The findings of genotype and allele distribution for the ER22/23EK and Tth111I polymorphisms in the GR gene in BA patients and the controls are presented in Table 1.

We found no statistically significant difference in the distribution of alleles and genotypes for the ER22/23EK polymorphism in the GR gene in patients with asthma and apparently healthy individuals (p = 0.126); however, we revealed a statistically significant difference in the distribution of genotypes for the Tth111I polymorphism in the GR gene in patients with asthma and apparently healthy individuals (p = 0.043). It should be noted that the GG-allele homozygotes were equally frequent in patients with asthma and apparently healthy individuals, while the minor allele homozygotes were 2.4 times more common among patients with asthma.

The findings of gender-stratified genotype and allele distribution for the ER22/23EK and Tth111I polymorphisms in the GR gene in BA patients and the controls are presented in Table 2.

The analysis of the distribution of genotypes and alleles for the ER22/23EK and Tth111I polymorphisms in the GR gene with regard to gender demonstrated no significant gender-related difference between the controls (p = 0.24; p = 0.185) and BA patients (p = 0.743; p = 0.75).

Table 3 represents the results of the analysis of BA risk regarding the ER22/23EK and Tth111I polymorphisms in the GR gene using binary logistic regression in four inheritance models.

The relative risk estimation of developing asthma regarding the ER22/23EK polymorphism did not show a statistically significant correlation in any inheritance model. The relative risk estimation of developing asthma in 4 models of inheritance with regard to the Tth111I polymorphism in the GR gene demonstrated a statistically significant correlation in the recessive model (p = 0.02). BA risk in recessive homozygotes was 2.69-fold higher vs. major allele homozygotes.

DISCUSSION

A sufficient amount of data was obtained on the large number of genes involved in the pathogenesis of asthma. Along with the genetic factors which determine the Th2-type of inflammation, directly define predisposition to atopy, and

Table 1. The ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor gene in patients with bronchial asthma and apparently healthy individuals

Genotype	BA patients, n = 553		Apparently healthy individuals, n = 95	
	n	%	n	%
		rs 6189/6190		
GG	496	89.7	79	83.2
AG	53	9.6	14	14.7
AA	4	0.7	2	2.1
		$\chi^2 = 4.14; P = 0.126$		
G-allele	94.6		90.5	
A-allele	5.4		9.5	
χ^2	3.87		1.88	
p	> 0.05		> 0.05	
		rs10052957		
CC	228	41.2	39	41.1
CT	240	43.4	50	52.6
TT	85	15.4	6	6.3
		$\chi^2 = 6.278; p = 0.043$		
C-allele	62.9		67.4	
T-allele	37.1		32.6	

Table 2. Distribution of alleles and genotypes for the ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor gene with regard to gender

Genotype	ER22/23EK polymorphism							
	Control group, n = 95				BA patients, n = 553			
	Men, n = 45		Women, n = 50		Men, n = 193		Women, n = 360	
	n	%	n	%	n	%	n	%
GG	40	88.9	39	78.0	174	90.2	322	89.4
AG	5	11.1	9	18.0	17	8.8	36	10.0
AA	0	0	2	4.0	2	1.0	2	0.6
	$\chi^2 = 2.9; p = 0.24$				$\chi^2 = 0.59; p = 0.743$			
G	94.6		87.0		94.6		94.4	
A	5.6		13.0		5.4		5.6	
	Tth111I polymorphism							
CC	17	37.8	22	44.0	79	40.9	149	41.4
CT	27	60.0	23	46.0	87	45.1	153	42.5
TT	1	2.2	5	10.0	27	14.0	58	16.1
	$\chi^2 = 3.37; p = 0.185$				$\chi^2 = 0.57; p = 0.75$			
C-allele	67.8		67.0		63.5		62.6	
T-allele	32.2		33.0		36.5		37.4	

Table 3. Association between the ER22/23EK and Tth111I polymorphisms in the glucocorticoid receptor gene and bronchial asthma risk

Model	P_{obs}	OR_{obs} (95% CI)	AIC
	ER22/23EK polymorphism		
Dominant	0.07	0.57 (0.31–1.07)	16.99
Recessive	0.22	0.34 (0.07–2.47)	18.8
Super-dominant	0.13	0.61 (0.33–1.19)	18
Additive	0.05	0.58 (0.35–1.02)	16.55
	TthIIII polymorphism		
Dominant	0.97	0.99 (0.63–1.54)	25.74
Recessive	0.02	2.69 (1.24–7.09)	19.22
Super-dominant	0.1	0.69 (0.44–1.07)	22.96
Additive	0.25	1.21 (0.88–1.68)	24.39

Notes: P_{obs} – observed p-value (not adjusted for covariates); OR_{obs} – observed odds ratio; CI – confidence interval; AIC – Akaike's information criterion.

control the degree of bronchial reactivity, a significant role in the development of asthma belongs to the mutations in the GR gene involved in BA pathogenesis which affect the effectiveness of glucocorticosteroid therapy [5].

Contrary to previous findings, a meta-analysis of studies evaluating the association of ER22/23EK (4 studies) and Tth111I (2 studies) polymorphisms in the GR gene with BA risk did not reveal any association in any genetic model [12]. This suggests no association between these polymorphisms and the risk of asthma. Panek M (2013) [5] evaluated the association between ER22/23EK polymorphism in the GR gene and asthma severity and symptom control and found no difference in the frequency of genotypes in asthma patients and the controls, as well as with regard to the level of disease control (p> 0.05). It should be noted that a previous study by Panek M. (2012) showed no association between the ER22/23EK polymorphic variant in the GR gene and the pathogenesis of asthma [5]. Similar data on the lack of correlation between ER22/23EK polymorphism and asthma were presented by Szczepankiewicz A [2]. The

obtained results revealed no significant difference in the distribution of alleles and genotypes for the ER22/23EK polymorphism in the GR gene in patients with asthma and apparently healthy individuals ($p = 0.126$); apart from that, we found no statistically significant association with BA risk in any model of inheritance.

Our study findings suggest the important role of the Tth111I polymorphism in the GR gene in BA pathogenesis by revealing the following: a statistically significant difference in the distribution of alleles and genotypes for this polymorphism in patients with asthma and apparently healthy individuals ($\chi^2 = 6.278$; $p = 0.043$); a 2.4-fold higher frequency of the minor allele homozygotes among BA patients vs. the controls; and a 2.69-fold higher BA risk in the carriers of TT-genotype for the Tth111I polymorphism in the GR gene vs. the major allele carriers. This was earlier demonstrated by Panek M (2013) in the Polish population: a strong correlation between TT genotype for the analyzed SNP and a high control level of severe allergic and non-allergic asthma [5]. This study revealed that Tth111I polymorphism in the GR gene played an important role in the development of asthma of both allergic and non-allergic nature, and correlated with the specific profile of asthma control according to ACT™. However, the study of Szczepankiewicz A. (2008) did not find any association of the Tth111I polymorphism in the GR gene with asthma and an increased need for high doses of inhaled GC. No gender-specific difference was observed in the distribution of genotypes and alleles for the ER22/23EK and Tth111I polymorphisms in the GR gene [2].

The inconsistency of these findings on the role of the ER22/23EK and Tth111I polymorphisms in the GR gene in the development of asthma can be explained by the clinical heterogeneity of this disease, a small sample of patients, different age of onset, and pathogenetic differences between different phenotypes of the disease. Identification of clinical phenotypes of asthma in recent studies demonstrated the genetic heterogeneity of the disease, assuming that their formation involves overlapping genetic factors and common pathogenesis mechanisms that determine a particular phenotype of the disease. Therefore, it is important to study gene polymorphisms in combination with clinical parameters that determine the phenotype of the disease.

CONCLUSIONS

We revealed no significant difference in the distribution of alleles and genotypes for the ER22/23EK polymorphism in the GR gene in patients with asthma and apparently healthy individuals ($p = 0.126$); apart from that, we found no statistically significant association with BA risk in any model of inheritance.

A statistically significant difference was observed in the distribution of genotypes for the Tth111I polymorphism in the GR gene in patients with asthma and apparently healthy individuals. The minor allele homozygotes were 2.4 times more common among BA patients vs. apparently healthy individuals.

BA risk was 2.69 times higher in the carriers of TT genotype for the Tth111I polymorphism in the GR gene vs. major allele carriers.

No gender-specific difference was observed in the distribution of genotypes and alleles for the ER22/23EK and Tth111I polymorphisms in the GR gene.

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REVIEW ARTICLE

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COMPETENCIES AND SKILLS NEEDED IN THE MANAGEMENT OF CHRONIC PATIENTS' NEEDS THROUGH TELECARE

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ABSTRACT

Aim: To identify the necessary competencies that future professionals must obtain in order to effectively manage patients with chronic conditions. We employed a multilayer review in PubMed, Scopus and Cochrane.

Materials and Methods: We applied three searches in PubMed, Scopus, and Cochrane using various terms in order to identify the necessary skills and competences needed for healthcare professionals to provide distance care in patients with chronic conditions. From the initial search, a total of 1008 studies were identified while 54 met the inclusion criteria and were retained for data extraction. After the review of the 54 studies, we grouped the proposed skills and competencies in eight major categories. Those groups were Clinical Knowledge, Critical Thinking Skills, Technological Skills, Clinical skills, Communication skills, Implementation skills, Professionalism and professional ethics, Evidence based Practice.

Conclusions: Although telehealth is gaining ground in healthcare practice and healthcare professionals possess the necessary knowledge and skills to provide safe, effective, and personalized care, additional specialized training is nevertheless required to provide telecare. Therefore, the integration of telehealth into various healthcare professions curricula - both at undergraduate and postgraduate levels – is required for the development of education and the dynamic development of healthcare.

KEY WORDS: chronic disease, competencies, telecare, telecare skills, telemedicine

INTRODUCTION

Chronic disease is characterized as a disease that has one or more of the following traits: it is permanent, leaves residual disability, is brought on by an irreversible pathological alteration, necessitates special patient training for rehabilitation, or the patient may be anticipated to need a lengthy period of supervision, observation, or care [1]. Chronic illnesses have a significant negative impact on people's lives because they increase mortality rates, reduce mobility, cause bad emotions, and reduce quality of life [2]. At least one in four American individuals had two or more chronic diseases in 2012, and half of all adults in the country had at least one [3]. The World Health Organization (WHO) reported in 2015 that chronic diseases cause 38 million deaths annually [4-6] and have turned into a public health problem [7]. Common chronic diseases include rheumatoid arthritis, diabetes, and hypertension. Worldwide, hypertension affects at least one billion people, and by 2025, that number is expected to rise to 1.56 billion [8].

Epidemiological survey findings suggest that there are 180 million diabetics globally, and that by 2030, this number could treble [9]. Rheumatoid arthritis affects 0.5% of adults worldwide, and it has risen to the top 10 chronic diseases in China. Additionally, the prevalence of rheumatoid arthritis in people in the United States ranges from 0.5% to 1.0% [10, 11]. Therefore, the aforementioned disorders should receive more attention. The use of remote care in the management and treatment of chronic patients is becoming more and more popular today. Telemedicine, monitoring, and health, per the European Commission (2008), can aid in the efficient use of medical services and cut down on hospital visits. Additionally, it may improve the standard of medical care, particularly for elderly and chronic patients. Finally, in order to ensure the sustainability of the services, the European Commission asserts that healthcare systems must address this issue through a collaboration encompassing patients, healthcare providers, payers, and the industry [12].

AIM

Although there are a growing number of studies focused on telemedicine for chronic disease management, however, there is no consensus on the effect of telemedicine on chronic disease management. Therefore, the aim of this study is to identify the necessary competencies that future professionals must obtain in order to effectively manage patients with chronic conditions.

MATERIALS AND METHODS

We employed a multilayer review in PubMed, Scopus, and Cochrane.

We performed three searches as follows:

In the first search, we tried to identify studies addressing the topic from an educational or healthcare professions perspective using the following algorithm: (Telemedicine[Title/Abstract] OR Telenursing[Title/Abstract] OR Remote consultation[Title/Abstract] OR telemed*[Title/Abstract] OR tele-med*[Title/Abstract] OR telenurs*[Title/Abstract] OR telecommunication*[Title/Abstract] OR mobile health[Title/Abstract] OR telerehabilitation[Title/Abstract] OR telecare[Title/Abstract] OR tele-care[Title/Abstract] OR tele-home[Title/Abstract] OR telehome[Title/Abstract] OR e-health[Title/Abstract] OR e health[Title/Abstract] OR tele-health[Title/Abstract] OR telehealth[Title/Abstract] OR remote assessment[Title/Abstract] OR remote treatment[Title/Abstract] OR remote consultation[Title/Abstract] OR telemonitor*[Title/Abstract] OR telehomecare[Title/Abstract] OR videophone[Title/Abstract] OR video conferencing[Title/Abstract] OR video visits[Title/Abstract] OR virtual visits[Title/Abstract] OR televisits[Title/Abstract] OR assistive technology[Title/Abstract] OR videotelephone[Title/Abstract] OR econsult[Title/Abstract] OR online therapy[Title/Abstract] OR Teleconferencing[Title/Abstract] OR m-health[Title/Abstract] OR mHealth[Title/Abstract] OR telephone monitoring alerts[Title/Abstract] OR telephone support[Title/Abstract] OR teleconference[Title/Abstract]) AND (Nursing Skills[Title/Abstract] OR Communication Skills Training[Title/Abstract] OR Social Skills Training[Title/Abstract] OR Social Skills[Title/Abstract] OR Communication Skills[Title/Abstract] OR Skill Acquisition[Title/Abstract] OR Skill Retention[Title/Abstract] OR Competency Assessment[Title/Abstract] OR Clinical Competence[Title/Abstract] OR Job Characteristics[Title/Abstract] OR best practices[Title/Abstract] OR health literacy[Title/Abstract] OR ehealth literacy[Title/Abstract] OR e-health literacy[Title/Abstract] OR computer skills[Title/Abstract] OR ICT knowledge[Title/Abstract]).

In the second search, we identified studies that addressed the topic from the patients' perspective. What patients need from health care professionals when they receive telecare services using the following algorithm : ((long-term conditions[Title/Abstract] OR chronic condition[Title/Abstract] OR Chronic Illnesses[Title/Abstract] OR chronic disease[Title/Abstract] OR chronic care[Title/Abstract] OR heart failure[Title/Abstract] OR asthma[Title/Abstract] OR respiratory diseases[Title/Abstract] OR respiratory conditions[Title/Abstract] OR diabetes[Title/Abstract] OR depression[Title/Abstract] OR dementia[Title/Abstract] OR arthritis[Title/Abstract] OR stroke[Title/Abstract] OR hypertension[Title/Abstract] OR blood pressure[Title/Abstract] OR cardiac diseases[Title/Abstract] OR cardiac conditions[Title/Abstract] OR COPD[Title/Abstract] OR pulmonary diseases[Title/Abstract] OR pulmonary conditions[Title/Abstract]) AND (patient needs[Title/Abstract] OR patient priorities[Title/Abstract] OR patient preferences[Title/Abstract])) AND (Nursing Skills[Title/Abstract] OR Professional Skills[Title/Abstract] OR Interpersonal skills[Title/Abstract] OR Communication Skills Training[Title/Abstract] OR Social Skills Training[Title/Abstract] OR Social Skills[Title/Abstract] OR Communication Skills[Title/Abstract] OR Skill Acquisition[Title/Abstract] OR Skill Retention[Title/Abstract] OR Competency Assessment[Title/Abstract] OR Clinical Competence[Title/Abstract] OR Job Characteristics[Title/Abstract] OR best practices[Title/Abstract] OR health literacy[Title/Abstract] OR ehealth literacy[Title/Abstract] OR e-health literacy[Title/Abstract] OR computer skills[Title/Abstract] OR ICT knowledge[Title/Abstract] OR soft skills[Title/Abstract]).

The eligibility criteria for inclusion studies in the review were: English publications; year of publication: 2011 to 2021; population – based sample; observational studies (cohort studies, patient-control studies, cross-sectional studies); Systematic reviews and meta-analyses AXIS was used for the quality assessment of the cross-sectional studies (20 questions) [13] and the CASP Checklist was used for the quality assessment of the cohort and case-control studies (12 questions for the cohort studies and 11 questions for the case-control studies) [14].

REVIEW AND DISCUSSION

From the initial search, a total of 1008 studies were identified: 672 studies at PubMed/Scopus and 336 studies at Cochrane. Of the 1008 studies, 112 were duplicates. Therefore, from the initial search, a total of 835 studied emerged. As it is shown in the flow chart (Fig. 1), of the 835 studies, only 54 met the inclusion criteria and were retained for data extraction. In Tables 1, 2, 3 the final set of skills and competencies that were identified is presented.

After the review of the 54 studies, we grouped the proposed skills and competencies in eight major categories, while skills and competencies that didn't fit in those were listed under the label others.

The first category that was identified was clinical knowledge skills, suggesting that healthcare professionals in order to adequately provide care from distance, must have observational skills, specialized knowledge, and be able to conduct a physical examination in a telehealth environment.

The second set of skills corresponds to critical thinking skills in this domain skills, such as skills in shared decision-making, decision making from distance, triage and clinical reasoning skills, knowledge of ways to evaluate the sources of information, and skills in identifying valuable sources for their information. These skills emerged from the review.

One very important set of skills that was identified are the technological skills that health care professionals must have. According to the results, healthcare professionals must have a wide range of skills regarding technology, from simple ones such as such as the use of the Internet and a personal computer to more complex ones such as the ability to check equipment for functionality, camera placement, lighting placement, and audio consideration. In addition, healthcare professionals must be able to train their patients for the use of such technologies.

Many studies reported specific clinical skills that healthcare professionals must have in order to provide care through telecare. Those skills are history-taking skills, case

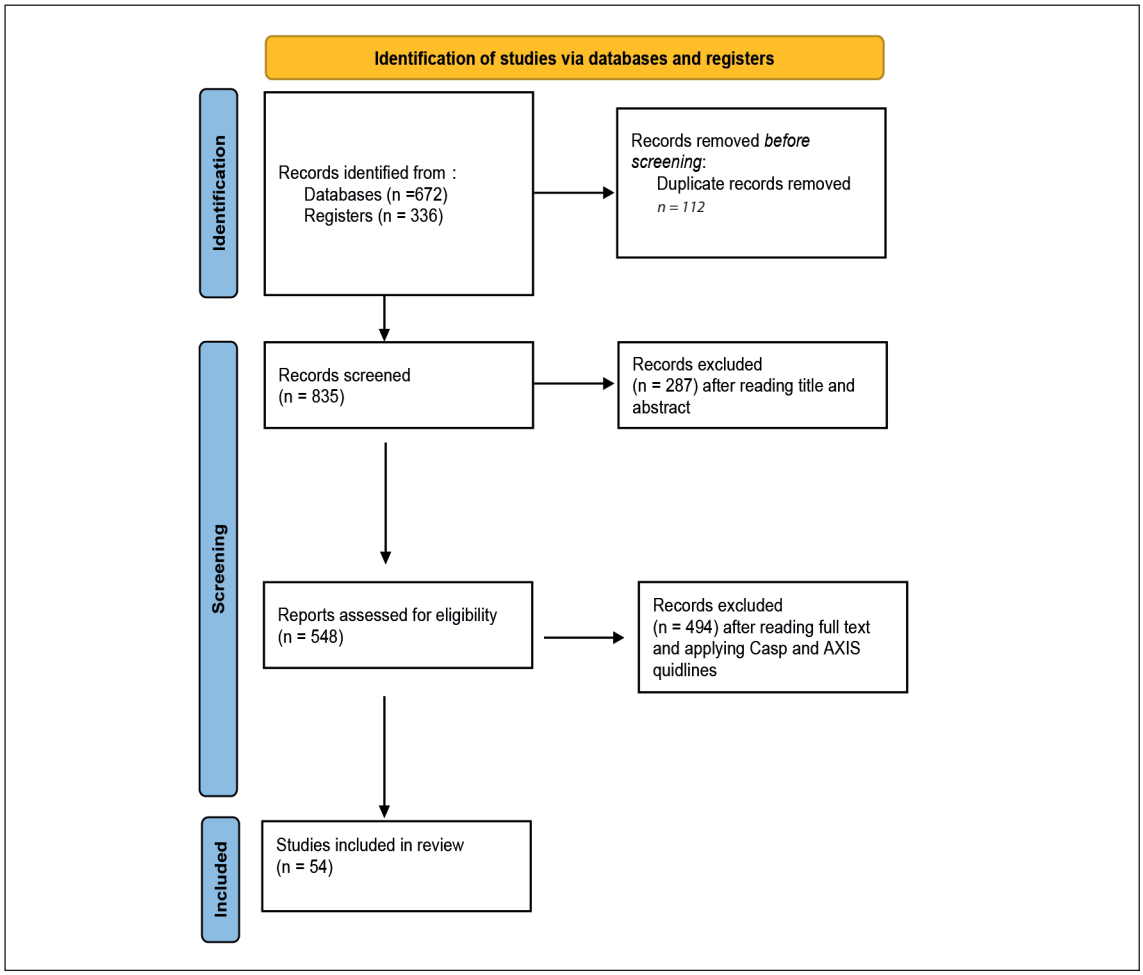

Fig. 1. Identification of studies for determining the necessary competencies for future health professionals in order to effectively manage patients with chronic conditions.

management skills, goal setting and action planning skill, personalized care skills. In addition, healthcare professionals must be able to interpret non-verbal and verbal expressions in the right way when videoconferencing and be able to compose a risk prevention plan to support patients' safe, independent living.

Similarly, traditional caring communication skills are considered to be very important in telecare settings. According to the literature, interpersonal and intercultural communication skills, trust-building skills, and listening skills are very crucial, as are skills such as empathy, emotional support, kindness, and therapeutic relationship-building skills. In addition, some specific telecare communication skills, such as telephone communication skills, telephone counseling skills, and remote consulting skills, have emerged.

Implementation skills were also identified as a category in the literature review. Studies are reporting that healthcare professionals must have motivational and documentation skills, as well as performance skills, such as the additional use of a preamble, checking the patient's current understanding of the illness, checking how well the news has been assimilated, and offering a second opinion. Moreover, they must be able to act for patient education and activation.

Another category of skills that emerged from the review were professionalism and professional ethics. For this category, skills studies state that healthcare professionals must have adequate knowledge of the laws and regulations concerning the protection and exchange of medical data, e.g., data protection, informed consent, and confidentiality. Moreover, they referred to knowledge of the laws, legislation, and regulations regarding licensure, billing, coding, and allowable practice. While others referred to best practices for conducting a virtual visit regarding appearance, professional dress, and background environment.

Another category that emerged was skills regarding evidence-based practice. In this category, skills such as insight into the reliability of health information on the web, knowledge of the most current information that may impact clinical practices, the rules, regulations, and financial aspects of telemedicine, and Skills to use patient medical records for clinical research were included.

Finally, we identified studies referring to other various skills such as cultural competencies, skills of teamwork and collaboration, social skills, and self-care skills, among others, that were very diverse and didn't fall into the aforementioned categories.

Table 1. Competencies and skills needed for chronic patients' distance care

a/a	Authors	Emerged skills
1 [15]	BARBOSA I et al. 2016	Abilities and communication skills
2 [16]	VAN HOUWELINGEN, CORNELIS TM, et al. 2016	**Clinical knowledge** What to do in case of an emergency Knowledge of telehealth technologies Knowledge of the limitations of telehealth Knowledge of how to deploy telehealth Knowledge of how technology can be used in sharing information with colleagues Knowledge of the laws and regulations concerning data protection, informed consent and confidentiality Knowledge of the potential benefits of telehealth Knowledge of how to collect health-related data Insight into the reliability of health information on the web Insight into the reliability of health information on the web Knowledge about what to do if the technology does not work **General skills** Able to think creatively to solve problems Coaching skills Ability to prioritize and switch quickly between different patients and different requests for help Protects the privacy of self and the patient in the use of telehealth technologies (compliance to ethical, legal and regulatory considerations) **Technological skills** Is able to train the patient to use the equipment Basic ICT skills, such as the use of the Internet and a personal computer Is able to check equipment for functionality Technological skills in the field of new technology Is able to use electronic health records **Clinical skills** Is able to combine clinical experience effectively with telehealth technology in decision-making Observation skills: interprets non-verbal and verbal expressions in the right way when videoconferencing Is able to use health-related data effectively in patient care Is able to compose a risk prevention plan to support patients' safe independent living Triage and clinical reasoning skills **Communication skills** **Implementation skills**
3 [17]	GOTTLIEB, A et al. 2022	**Knowledge** Of the most current information that may impact clinical practices, the rules, regulations, and financial aspects of telemedicine
4 [18]	WAHEZI, SE., et al. 2020,	Observation skills Clinical skills
5 [19]	ALQAHTANI, MM. J, et al. 2021,	Cultural competency Awareness of ethical issues and actions that arise in the context of providing medical care remotely
6 [20]	MOSS RJ, et al. 2019	Digital health literacy
7 [21]	BRAHMBHATT D et al. 2021.	Knowledge of techniques required for providing routine care with the use of digital technologies Basic principles of telemedicine communication technical skills, and physical examination Data management skills Clinical skills: decision making from a distance
8 [22]	BURG, F et al. 2021	Digital health literacy
9 [23]	ELLIOTT, T et al. 2022	Comfort with technology on the part of the clinician Knowledge of best practices for conducting a virtual visit regarding Appearance, Professional Dress, and Background Environment Interpersonal and communication competencies (Building rapport, Active listening, Sharing information/providing guidance, Shared decision-making)
10 [24]	BARBABELLA, F, et al. 2017.	Digital skills
11 [25]	SHEA, K et al. 2021	Communication skills
12 [26]	LOWE, JT, et al. 2021	Technical knowledge including camera placement, lighting placement, and audio considerations. Observation skills and clinical skills for Conducting a History, Acquiring Vital Signs and Completing a Physical Exam

13 [27]	JABAREEN et al. 2020	Computer and health informatics skills Skills to monitor patients' diagnosis and treatment, including access to clinical findings Skills to use shared hospital services Skills to use patient medical records and to manage electronic patient data Skills to use patient medical records for clinical research Skills to use coding standards
14 [28]	DASHTI, et al. 2017	E-Health literacy (traditional literacy, health literacy, information literacy, scientific literacy, media literacy and computer literacy) Knowledge of ways to evaluate the sources of information and skills in identifying valuable sources for their information
15 [29]	WAMSLEY, M, et al. 2021,	Knowledge of the specific the telehealth platform and skills with specific technology platforms Telehealth competencies Technical skills needed to operate equipment and software, including troubleshooting difficulties Professionalism in telehealth, including review of informed consent and patient privacy Telehealth communication skills Physical examination skills in the telehealth environment Knowledge regarding affordances and limitations of telehealth visits, including The potential for telehealth to increase disparities in care
16 [30]	JIMÉNEZ-RODRÍGUEZ, D et al. 2022	Humanization competencies.
17 [31]	MOON, S et al., 2021	Professionalism Communication skills Physical examination skills in the telehealth environment Technical knowledge including camera placement, lighting placement, measures to prevent sources of potential disruptions
18 [32]	ESPER, GJ, et al. 2020	Knowledge regarding telehealth (benefits of telehealth, basic applications, definitions, types of telehealth, licensing requirements) Knowledge of the laws, legislation and regulations regarding licensure, billing, coding, and allowable practice Knowledge regarding pre-, intra-, and post-telehealth visit processes
19 [33]	BOARDMAN, D, et al. 2021	Communication skills (including information gathering, relationship development, and patient education and counseling) Patient education, activation, and satisfaction skills Telemedicine skills (sound, screen visualization)
20 [34]	BOWE, et al. 2020, .	Technical knowledge including camera placement, lighting placement, positioning Virtual examination skills and decision making from distance
21 [35]	JOHNSON, C et al. 2015	Communication skills Interpersonal skills Competences for relationship-building
22 [36]	BANERJEE, SC, et al. 2021	Communication skills: establishing the clinician-patient relationship and creates rapport with the patient, setting an agenda for the tele-oncology visit, . Skills such as acknowledge, validate, normalize, and encourage expression of feelings
23 [37]	BRUNNER, M et al.2018.	Technical and informatics literacy Health informatics competencies E-Health-communication: Health information management Communication Professionalism Information systems and technologies Patient focus Health analytics Digital technologies, systems, and policies Clinical practice and applications System and technology implementation
24 [38]	ELHADI, M et al. 2021	Computer, software, information technology and internet skills Knowledge and understanding of telemedicine
25 [39]	ALKUREISHI, MA, et al., 2021	Patient-centered telemedicine competencies Virtual visit communication skills Ability to perform a comprehensive video-based physical exam
26 [40]	ROBERTS, LC et al. 2021	Communication skills

27 [41]	SLOVENSKY et al. 2017	Communication skills using digital technologies Technology literacy and ability to use mobile technologies Cultural competence Knowledge of mHealth products and services Knowledge of regulatory and compliance issues Cinical/professional knowledge and skills
28 [42]	KIRCHBERG, J et al. 2020,	E-Health literacy Knowledge about the safe and secure implementation of technologies in routine clinical practice
29 [43]	COLEMAN, C. 2020	Communication skills-clear communication
30 [44]	LIU, XIAO, et al. 2007	Communication skills Ability to express empathy in telemedicine consultations
31 [45]	LAWRENCE, K et al., 2020	Technical proficiency (such as screen location, room lighting, audio adjustments, and computer system optimization) Information competency skills including virtual information gathering, (history, collateral information collection) Clinical skills- physical exam skills in virtual environment Interpersonal communication skills, both verbal and nonverbal
32 [46]	TRAMONTANO, C et al. 2021	Digital skills including knowledge and personal efficiency competencies Self-care skills (Self Care competency) representing the ability of workers to successfully and positively manage work-life boundaries that digital technologies provide by allowing them to access work at any time and from any location. Remote social skills (Social & Relational Competency) demonstrating the ability to manage and promote social relationships from a distance. Remote emotional skills (Emotional Competency) reflecting worker's capabilities to manage and act on their emotions when working remotely.
33 [47]	BJÖRNDELL, C et al. 2021	Remote consultation and communication skills Clinical experience Skills for virtual examination

The aim of the present study was to identify skills and competencies that healthcare professionals are needing in order to meet the healthcare needs of chronic patients through telecare. According to the results of the present review, healthcare professionals must have a variety of skills ranging from traditional clinical-related skills to technological, cultural, and communication skills.

In the 21st century, during the time of the pandemic, telemedicine contributes to the quick access of the individual to health care services. It enables patients to have follow-up and, at the same time, eliminates "contact" between patients, nurses, and the community. It offers the possibility of 24-hour daily communication and evaluation of the indications of the patients, who have not been scheduled for hospitalization based on the symptoms they display, with the doctors. Communication can be implemented using smart phones or computers that have a camera, thus enabling continuous patient care. It is also possible to take a thorough medical history without transporting the patient to a health care facility and to implement management algorithms for suspected cases, hence reducing the likelihood of exposing other patients and health care personnel to cases [69]. Chronically ill people, the elderly with limited mobility, and patients in remote areas are some groups of people that are most likely to benefit from the applications of telecare. According to Haleem at al. (2021), telemedicine or telehealth can contribute to chronic disease management and prescription compliance, provide distant healthcare services, and provide care for acute and severe conditions. Moreover, telehealth applications such as wearable devices can help patients participate in monitoring and updating their health status. Although telemedicine has a wide range of applications and can provide emergency care for critical and non-critical situations it is usually used for treating patients suffering from chronic conditions [70].

The results of this review indicated a wide range of skills that future healthcare professionals must have in order to provide adequate distant care. The skills were grouped into nine domains, consisting of technological, digital, clinical, and communication skills among others. According to a recent review conducted by Fisk, Woolham, and Steils, there are six sets of skills that healthcare professionals must have regarding telecare. Those sets are understanding the role of telecare services, having the right ethos (to be able to recognize specific healthcare needs), having confidence in digital technology (the staff must be able to support patients as well), having confidence in data and information (the staff must be able to understand the importance of digital health and the digitalization of healthcare services), respecting regulations and standards (to understand regulations that apply in telecare procedures), and understanding particular conditions and service options (the staff must be aware of long-term conditions that are very common in the population and how to address them [71]. Although the review by Fisk et al. covers a lot of skills, little is

Table 2. Studies focusing on needs according Patients perspective

		Patient needs	skills
34 [48]	Lins, S et al. 2014	Barriers and facilitators for successful implementation of telephone counselling: Because counsellors are unable to witness caretakers' reactions and comprehend how they digest their recommendations, the spatial distance can be a concern. Caregivers and counsellors both agree that getting to know one another before to counseling is critical. Carers describe their need for a counsellor they can confide in with concepts like 'close friend', 'confide' or 'to be private'. Counsellors face difficulties to provide telephone counseling to carers who do not know the national language. After counseling sessions, counselors indicated the need for debriefing with colleagues, especially if they had to deal with challenging situations. The individualization of counselling was praised by both carers and counsellors.	Interpersonal communication skills Trust-building skills Listening skills Observation skills Cultural competency
35 [49]	Vaona, A., et al. 2017	Telephone consultation skills are part of a larger set of remote consulting skills that are becoming increasingly important as more medical care is offered remotely with the use of technology.	History-taking and case management skills Remote consulting skills Telephone communication skills Active listening and advising skills
36 [50]	Kew, K et al 2017.	Shared Decision Making has certain advantages over control.	Skills for Shared Decision-Making Communication skills (general and active listening skills, verbal and written language skills, expressive and receptive non verbal skills, attitudinal skills including the ability to listen to, understand, and discuss a different viewpoint, perspective, or value than one's own, while preserving respect for the patient's right to make his or her own decisions.
37 [51]	Coulter, A et al 2015.	Personalized care planning for persons with long-term conditions improves some indices of physical and psychological health, as well as people's ability to self-manage their condition.	Skills in shared decision-making, behaviour change, coaching, goal setting and action planning*
38 [52]	Ivynian, SE et al. 2020	Patient communication needs and preferences Need for credible, tailored, clear and detailed information Preference for face-to-face information delivery Need for plain language Need for positive and supportive relationship, incorporating trust, Compassion, flexibility and effective two-way communication be-Tween patient and provider Need for provider's openness to discussion Need for practical self-care information Need for information about how to cope emotionally	Communication skills
39 [53]	Noordman, J et al., 2020	Patient's instrumental need/need to know and understand Patient's affective need/need to feel known and understood (need for receiving hope, reassurance, empathy, and appreciation and discussing emotional coping)	Empathy Communication skills Kindness Care and attention
40 [54]	Kaplan, & Price, 2018	Patients' need for shared decision-making and need to participate in treatment decisions and to express their expectations and concerns	Communicative skills Conversation skills Empathy Competence and confidence in pro-Viding patients with information for Shared decision-making
41 [55]	Taylor, PC et al., 2021	Need for patient support programs including educational materials about the disease and its therapy	Patient communication capabilities Specialized knowledge

42 [56]	Canaud B etal, 2021	Need for personalized treatment Need for a patient-centered care approach by including various patient dimensions (i.e. metabolic, perception, social, family)	Personalized care skills Communication skills Motivational skills Documentation skills Clinical skills Therapeutic relationship-building Skills
43 [57]	Mattukat, K et al.,2019	Need to receive adequate information on the disease and treatment options not all patients prefer being involved in medical decision-making	Communication skills Therapeutic relationship-building Skills
44 [58]	Fujimori, M et al., 2014		Communication skills Confidence in communicating with patients Emotional support skills Empathic skills, including the use of silence and accepting a patient's expression of emotions performance skills, such as the additional use of a preamble, checking the patient's current understanding of the illness, checking how well the news has been assimilated, and offering a second opinion
45 [59]	Sarkar, U et al., 2008	Patient's interest in receiving self-management support, with preferences for modes of delivery of self-management support varying by race/ethnicity, language proficiency, and self-reported health literacy	Therapeutic relationship-building Skills
46 [60]	Dowsett, S et al., 2000	Preference for a patient-centred approach to the consultation, particularly when the patient has a poor prognosis	Empathy Openness Reassurance Interpersonal skills Communication skills Non-verbal skills Therapeutic relationship-building Skills
47 [61]	Aerts, N., et al. 2020	Patients' need for the healthcare provider to invest in health advocacy and individualising care Patients' need for time to adapt to the new situation by gradually introducing the practice nurse role and encouraged by the already established trust relationship with their general practitioner Need for patient-centred and collaborative care	Social skills Therapeutic relationship-building Skills Empathic skills to provide individualized care Communication skills Skills of teamwork and collaboration
48 [62]	Dwamena, F et al., 2012	Promotion of patient-centred care skills in the clinical consultation Adding explicit patient and/or provider instruction in disease-specific management skills to patient-centred care skills	Patient-centred care skills Disease-specific management skills Communication skills
49 [63]	Coulter, A, , R. 2015	Personalized care planning	Skills in shared decision-making Communication skills Empathic skills to provide individualized care Personalized care skills
50 [64]	Rolfe, A., et al. 2014	Trust Two studies trialled physician training interventions to improve behaviours known to be associated with trust.	Humanistic skills Communication skills Technical competence Professionalism skills Cultural competency Confidentiality
51 [65]	Attridge, M., et al 2014	Culturally appropriate health education improves glycemic control and awareness of diabetes and healthy lifestyles in the short to medium term.	Cultural competency Intercultural communication skills

52 [66]	Horvat, L., et al 2014	Five studies looked at how cultural competence training for health professionals affected patient outcomes.	Cultural awareness, cultural knowledge and cultural skills Intercultural communication skills Inter-personal skills to help people work with others, such as advanced communication, negotiation or collaboration Intra-personal skills to help individuals cope with situations. This can include cultural self-assessment and reflection, and deconstructing stereotypes. Collaboration skills Non-verbal communication skills Skills to anticipate and deal with client resistance
53 [67]	Farooq, S., et al. 2017	In the case of illnesses like schizophrenia, which has the potential to cause substantial life disruption for both people with schizophrenia and their caretakers, good diagnosis communication can improve treatment planning, compliance, and patient outcomes.	Communication skills and skills to deliver bad news
54 [68]	Kalra, N. et al., 2021	Overall, healthcare providers (HCPs) may benefit from Intimate partner violence (IPV) training for outcomes that are precursors to behavior change.	Interviewing skills Violence screening skills Advocacy skills Interpersonal skills such as the ability to listen actively, communication skills, awareness and critical thinking.

Table 3. Grouping of Skills and competencies

	Category of Competencies	Skills & Competencies (reference)
1	Clinical Knowledge	Observation skills (32, 40) Specialized knowledge (55) Physical examination skills in the telehealth environment (45, 48, 37, 53, 59)
2	Critical Thinking Skills	Skills in shared decision-making (65, 63) Openness (60) Decision making from distance (48) Triage and clinical reasoning skills (16) Insight into the reliability of health information on the web (16) Is able to combine clinical experience effectively with telehealth technology in decision-making ? (16) Knowledge of ways to evaluate the sources of information and skills in identifying valuable sources for their information (42)
3	Technological Skills	Is able to train the patient to use the equipment (356) Basic ICT skills, such as the use of the Internet and a personal computer (16, 41) Is able to check equipment for functionality (16) Knowledge about what to do if the technology does not work (16, 43) Technological skills in the field of new technology (44) Is able to use electronic health records (16) Digital health literacy (34) Knowledge of techniques required for providing routine care with the use of digital technologies (35) Basic principles of telemedicine communication, technical skills, and physical examination (35) Comfort with technology on the part of the clinician (37) Digital Skills (38) Technical knowledge, including camera placement, lighting placement, and audio considerations (54, 59, 63, 59). E-Health literacy (traditional literacy, health literacy, information literacy, scientific literacy, media literacy and computer literacy) (43, 55) Knowledge regarding telehealth (benefits of telehealth, basic applications, definitions, types of telehealth, Licensing requirements) (46, 47, 52) Knowledge regarding pre-, intra-, and post-telehealth visit processes (46) Technical and informatics literacy health informatics competencies (51, 52) Capabilities -clinical-personal- eHealth-communication: Health information management (Recording and storing health information in electronic systems, ensuring data quality, and information governance) (51) Information systems and technologies (Using information systems and technologies to support routine clinical care, business processes, and patient-centered service provision) (51, 56) Knowledge of mHealth products and services (41)

4	Clinical skills	Observation skills (62) History-taking (59, 63) Case management skills (63) Goal setting (65) Action planning (65) Care (67) Attention (67) Personalized care skills (56, 63) Clinical skills (56) Empathic skills to provide individualized care (61, 63) Observation skills: interpreting non-verbal and verbal expressions in the right way when videoconferencing (16) Is able to use health-related data effectively in-patient care Is able to compose a risk prevention plan to support patients' safe, independent living Patient focus (Patient empowerment, use of technology for self-management and wellness, patient eHealth literacy, and education) (51) Clinical experience (51)
5	Communication skills	Interpersonal communication skills (23, 35, 48, 66) Intercultural communication skills (65, 66) Trust-building skills (48) Listening skills (48, 50) Telephone communication skills (49) Active listening skills (49, 50) Verbal and written language skills (50) Expressive nonverbal skills (45, 50) Receptive nonverbal skills (45, 50) Communication skills (23, 24, 31, 35, 36, 37, 39, 40, 41, 43, 44, 45, 47, 52, 53, 54, 56, 57, 58, 61, 62, 63, 64, 67) Skills to deliver bad news (67) Violence screening skills (68) Interviewing skills (68) Advocacy skills (68) Empathy (53, 54, 60) Kindness (44, 53) Conversation skills (54) Competence and confidence in providing patients with information for shared decision-making (54) Patient inter personal communication capabilities (45, 55) Therapeutic relationship-building skills (35, 56, 57, 59, 60, 61) Confidence in communicating with patients (58) Empathic skills, including the use of silence and accepting a patient's expression of emotions (58) Reassurance (60) Interpersonal skills (45, 60) Interpersonal skills such as the ability to listen actively, communication skills, awareness, and critical thinking (68) Intra non-verbal skills (60, 66) Patient-centered care skills (62) Intra-personal skills to help individuals cope with situations (66) Attitudinal skills, including the ability to listen to, understand, and discuss a different viewpoint, perspective, or value than one's own, while preserving respect for the patient's right to make his or her own decisions (50) Skills to anticipate and deal with client resistance (66) Skills such as acknowledging, validating, normalizing, and encouraging the expression of feelings (36) Consulting skills (47, 49) Telephone counseling (48, 49) Remote consulting skills (49) Advisory skills (49) Coaching (51) Emotional support skills (46, 58)
6	Implementation skills	Motivational skills (56) Documentation skills (56) Performance skills, such as the additional use of a preamble, checking the patient's current understanding of the illness, checking how well the news has been assimilated, and offering a second opinion (58) Patient education, activation, and satisfaction skills (33)

7	Professionalism and professional ethics	Knowledge of the laws and regulations concerning the protection and exchange of medical data, e.g., data protection, informed consent, and confidentiality (2) Professionalism (Critical appraisal, evidence-based practice, eHealth literacy, continued professional development, ethical use of information, and management and leadership) Knowledge of the laws and regulations concerning the protection and exchange of medical data, e.g., data protection, informed consent, and confidentiality (2, 31, 42) Awareness of ethical issues and actions that arise in the context of providing medical care remotely (5) Knowledge of best practices for conducting a virtual visit regarding appearance, professional dress, and background environment (9) Professionalism in telehealth, including review of informed consent and patient privacy (15) Knowledge of the laws, legislation, and regulations regarding licensure, billing, coding, and allowable practice (32) Professionalism (critical appraisal, evidence-based practice, eHealth literacy, continued professional development, ethical use of information, and management and leadership) (37) Knowledge of regulatory and compliance issues (41) Clinical/professional knowledge and skills (41)
8	Evidence based Practice	Insight into the reliability of health information on the web (2) of the most current information that may impact clinical practices, the rules, regulations, and financial aspects of telemedicine (3) Skills to use patient medical records for clinical research (13)
9	Others	Cultural competency (5, 41, 48, 65) Cultural awareness (66) Cultural knowledge (66) Cultural skills (66) Behavior change (51) Social skills (46.61) Skills of teamwork and collaboration (61) Collaboration skills (66) Disease-specific management skills (62) Own management skills (64) Humanization competencies (optimism to generate positive future expectations, sociability to relate to others appropriately with assertiveness and empathy, emotional understanding to empathize cognitively with others, placing ourselves in their place, self-efficacy to manage successfully complex and stressful situations, and affection to empathize emotionally with the affective state of another person) (16) Self-care skills (Self Care competency) representing the ability of workers to successfully and positively manage work-life boundaries that digital technologies provide by allowing them to access work at any time and from any location (46)

written about soft skills such as communication and critical thinking. It is widely accepted that telehealth providers require high-level communication skills in order to balance the lack of physical presence [72]. According to an expert consensus paper regarding telehealth skills needed by healthcare professionals, communication skills are very important. Healthcare professionals who deliver telecare, among others, must be able to create rapport, build relationships, and work effectively with patients, families, and caregivers. In addition, they must refer to and collaborate with other healthcare professionals and support other categories of staff that are engaging in the telecare procedure. Moreover, they must demonstrate clarity and empathy to create a connection in the absence of in-person care [73].

Taking inconsideration of the results of the present literature review there is a growing need for educational competence of healthcare workers on the subject of telecare to be imperative, while placing the relationship of educational competence and educational readiness of healthcare workers as a means by which they can be integrated into the order of things imposed by the expertise of telecare. In fact the training of healthcare professionals must take place in campus in their student years. According to a recent study among undergraduate students in Bangladesh, awareness and knowledge among the people are one of the most important parameters to improve the quality of telemedicine. In fact, according the results of the aforementioned study knowledge of telemedicine and telecare systems can enhance the ability of students to adapt in such new technologies and they conclude that there is an ongoing be well trained and more awareness should be created [74]. According to Khan et al. [75], the lack of knowledge has been identified as the main factor that discourages doctor and nurses to implement telecare in their practice. While the same researchers are highlighting the necessity of adaptation of hospital staff to new technologies and the positive effect that workshops could to knowhow of modern IT devices and technology systems. The necessity and the value of education and awareness of healthcare professionals have been highlighted from WHO since 1997, yet they are too many steps to take in order to provide digital care [76, 77].

CONCLUSIONS

The COVID-19 pandemic has led to the use of new ways, some already existing, in the workplace, communication, commerce, education and even treatment and patient care. Telecare is emerging as the means to improve quality and access to health care, facilitating the entry of more sustainable models for health care. It enables individuals to live independently and actively, positively impacting their well-being and quality of life. Telecare applications are providing timely, efficient and quality management of patient care. It also gives these people a sense of safety and security, since if their condition changes, the care team will be "present" to help them. Although telehealth is gaining ground in healthcare practice and healthcare professionals possess the necessary knowledge and skills to provide safe, effective, and personalized care, additional specialized training is nevertheless required to provide telecare. Therefore, the integration of telehealth into various healthcare professions curricula - both at undergraduate and postgraduate levels - is required for the development of education and the dynamic development of healthcare. Healthcare organizations as well as university must take proper planning and training for telecare use seriously in order to benefit healthcare personnel. Staff should receive training as part of the telecare implementation processes in order to support the information, skills, and attitudes needed for new ways of working and enable quality and safety in telecare practice. To create positive patient-professional connections when providing treatment remotely, telecare training should be practical and promote a general patient-centered approach to care. The need for things like verbal communication, conducting physical exams via telehealth, and competent clinical reasoning in telemedicine assessment and therapy was emerged. Thus, is crucial to prepare and support graduates to utilize telehealth in the workplace.

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REVIEW ARTICLE

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THE RELATIONSHIP BETWEEN PSYCHOSOCIAL WORK ENVIRONMENT AND NURSES' PERFORMANCE, ON STUDIES THAT USED THE VALIDATED INSTRUMENT COPENHAGEN PSYCHOSOCIAL QUESTIONNAIRE (COPSOQ): AN EMPTY SCOPING REVIEW

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ABSTRACT

Aim: This study was to map the relationship between psychosocial work environment and nurses' performance, on studies that used the Copenhagen Psychosocial Questionnaire (COPSOQ).

Materials and Methods: The review intended to answer following questions: 'Can COPSOQ screen completely psychosocial risks of nurses' work environment?' 'Which of these dimensions affect more nurses psychosocial world? A scoping review was developed guided by the JBI methodology and using PRISMA-ScR.

Conclusions: Hospital workers, and in particular nurses, are exposed to different risk factors with the most important being psychosocial risks. These arise from problematic work planning, organization and management, as well as from an unhealthy social context of work and may lead to negative psychological, physical and social outcomes. The review highlighted the need for further research using the entire COPSOQ questionnaire in order to fully study the psychosocial risks that nurses face in their work environment.

KEY WORDS: COPSOQ, Nurses, Psychosocial Risks

INTRODUCTION

According to World Health Organization, health is defined as "the state of complete physical, mental and social well-being and not simply the presence of illness and disability" [1]. Therefore, health is a complex, not only biological but also psychosocial phenomenon, which in addition to its self-evident biological dimensions includes the concepts of well-being and functionality. Work has an impact on health in many ways, since stress at work is the second most common health problem, affecting one third of workers in the European Union [2, 3].

Healthcare workers are often exposed to physical and psychosocial dangers that de-rived from work-related circumstances [4]. More specifically, the profession of nursing considered to be one of the most hazardous jobs, as nurses spend most of working time helping and serving patients. This includes a lot of hours walking, bending, stretching, and standing, making them vulnerable to physical injury. But as mentioned before, nurses experience a lot of psychosocial risks fulfilling their duty [5, 6]. Such factors may be related to aspects of the planning, organization and management process of work, lack of supportive relationships, job insecurity or even and in the culture of a business [7-9]. The interaction between personal/individual (characteristics, abilities and needs of the employee) and organizational factors (workplace organization, environmental and organizational conditions, work content) is what leads to the development of psychosocial risks. Psychosocial risks arise from problematic work planning, organization and management, as well as from an unhealthy social context of work and may lead to negative psychological, physical and social outcomes such as work-related stress, burnout or depression[10].

Some examples of working conditions that may lead to psychosocial risks are, excessive workload, conflicting demands and ambiguities regarding the role of the worker, lack of participation in decision-making affecting the worker and lack of influence on how work is carried out, poor management of organizational change, job insecurity, ineffective communication, lack of support from management or colleagues, psycho-logical and sexual harassment, violence perpetrated by third parties. Exposing workers to these risks usually leads to increasing levels of work-related stress, which negatively affects their

skills and effectiveness in their work duties, often leading to the occurrence of physical/mental illness, mistakes or even accidents[10-12]. Employees experience anxiety attacks when their job demands are excessive and exceed their ability to cope with them [13]. In addition to mental health problems, workers suffering from prolonged stress are at risk of developing serious physical health problems, such as cardiovascular disease or musculoskeletal problems [14, 15].

At the organization or enterprise level, negative consequences may include poor overall business performance, increased rate of absence from work, typical presence at work (cases of employees appearing at work while ill and unable to function effectively) and increased accident and injury rates [16, 17].

Hospital workers, and in particular nurses, are exposed to different risk factors with the most important being psychosocial risks, including Work-Strain [18], Bullying [19], Burn-Out [20], Emotional Strain [21], Work Life Balance [22], Job Satisfaction [23], Intention to Leave [24], Mental Health Issues [20] and other factors lurking in the modern work environment.

The psychosomatic health and balance of employees in the health environment has been found to be related to the safety and quality of the health services provided [25]. In order to ensure the quality of health services provided and the safety of patients and employees, the formation of appropriate psychosocial working conditions is a priority.

AIM

The present review aims to map the relationship between psychosocial work environment and nurses' performance, on studies that used the Copenhagen Psychosocial Questionnaire (COPSOQ). The review intended to answer following questions: 'Can COPSOQ screen completely psychosocial risks of nurses' work environment?' 'Which of these dimensions affect more nurses' psychosocial world?'

MATERIALS AND METHODS

STUDY DESIGN

The literature review showed a lot of studies on the subject, looking at it from different perspectives. For this reason we started this review, following the preferred reporting items for scoping reviews (PRISMA-ScR) and was guided by the methodology proposed by JBI to adequately conduct scoping reviews [26-28].

ELIGIBILITY CRITERIA

In an initial phase, inclusion and exclusion criteria were defined for the review can be find in Table 1. More specifically, the inclusion criteria included papers which mentioned to all educational levels of nurses' psychosocial working environment and were cross sectional questionnaire surveys, cohort and longitudinal studies. The surveys used COPSOQ questionnaire (I, II, III, sort, medium, long), were published between 2000 and 2021, the language was English and provided free access to the full text. The period was defined to fulfill all studies that used COPSOQ questionnaire.

The exclusion criteria were defined as: documents that did not use COPSOQ questionnaire, literature reviews, qualitative or mixed studies, the published period was no between 2000 and 2021 and were not provided free access in English language.

DATA COLLECTION

The second phase was to search and pick all these records that suit the review. As described in Table 2, all records found were extracted through Google Scholar, Med-line databases, COPSOQ International Network and Science Direct. These databases were chosen because they are suitable for a review in the area nursing care. To answer the research questions certain keywords were used, that were common to all databases. More specifically these keywords were: COPSOQ, COPSOQ Nurses, COPSOQ II Nurses, COPSOQ III Nurses.

DATA PROCESSING AND ANALYSIS

Three researchers took part in searching the databases. The process was held in-dependently by each researcher in order to maintain the integrity of the review. Initially, two of them read only titles and abstracts. The third one worked as a consultant and intervened when no consensus was reached. All documents that selected were read in full and an excel document, which was created including following: author, year of publication, location, methodology, aim, period, sample size, instruments, results. This information was essential selecting and analyzing the content of the documents.

Table 1. Eligibility Criteria

	Eligibility Criteria	
	Inclusion	**Exclusion**
Concept	Psychosocial work environment and nurses' performance	Documents that did not identify psychosocial work environment and nurses' performance
Context	All nurses are included regardless of educational level and workplace	Documents that included and other health care workers (doctors, dentists etc.) and had non-obvious results
Type of text	Cross-sectional questionnaire surveys,cohort studies, longitudinal studies	Literature reviews, qualitative or mixed studies
Type of Questionnaire	Surveys that use COPSOQ (i, ii, iii, sort, medium, long version)	Surseys that use part of COPSOQ
Language of publication	English	Documents that were not in English

Table 2. Indexed terms selection strategy

Database	Indexed Terms	Records Retrieved
Google Scholar	COPSOQ	6.460
	COPSOQ Nurses	3.070
	COPSOQ ii Nurses	2.160
	COPSOQ iii Nurses	1.430
PubMed	COPSOQ	84
	COPSOQ Nurses	14
	COPSOQ ii Nurses	3
	COPSOQ iii Nurses	2
COPSOQ International Network	COPSOQ	1.628
Science Direct	COPSOQ	164
	COPSOQ Nurses	55
	COPSOQ ii Nurses	30
	COPSOQ iii Nurses	8
Total		14.090

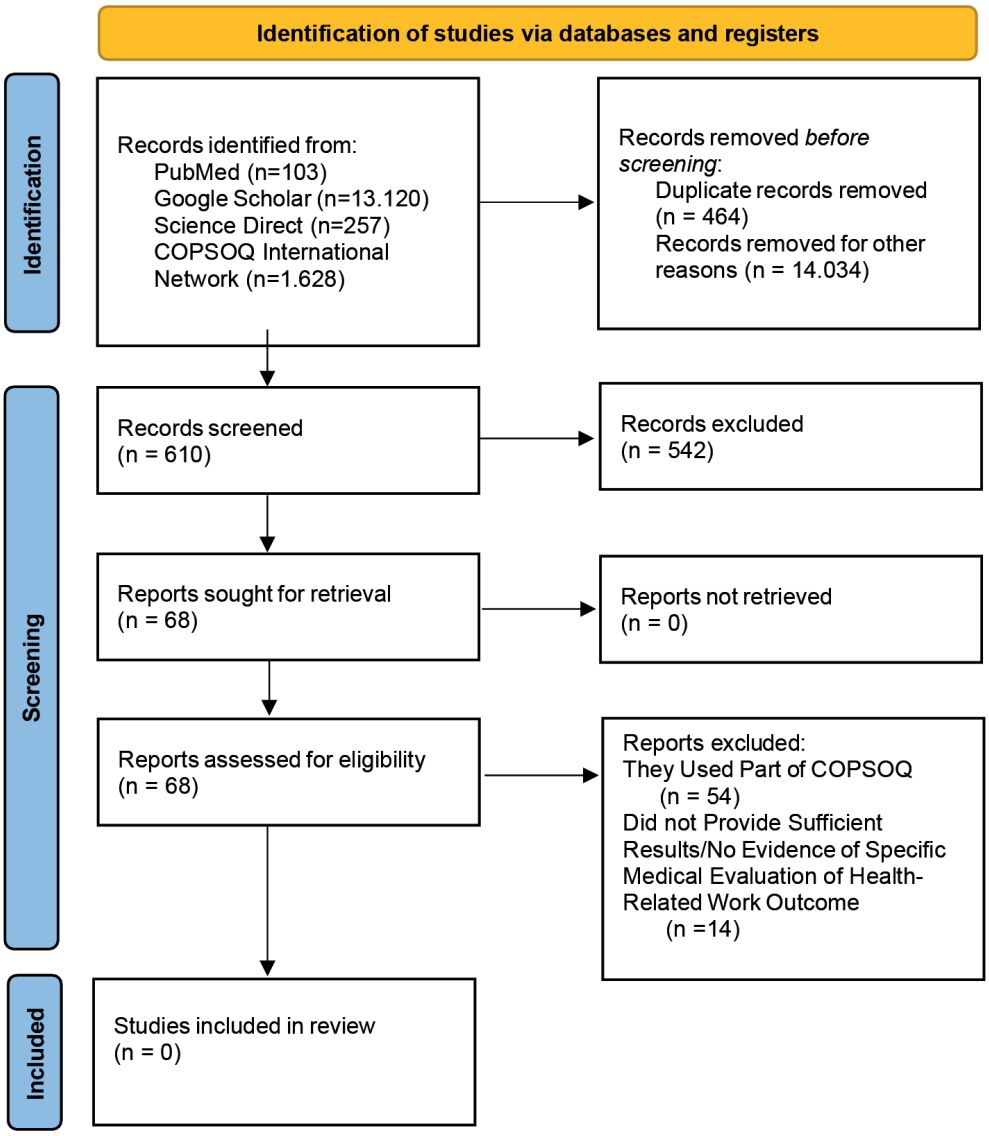

Fig. 1. Flow Diagram.

REVIEW AND DISCUSSION

From the research 15.108 articles were found in the databases, as show in Figure 1. The first phase was to remove duplicate studies and the documents that did not fulfill inclusion criteria. The number of articles that excluded were 14.498. At the second phase 542 articles were excluded after careful reading because they did not meet the inclusion criteria. At third face 68 remaining documents were read, from whom 68 were excluded based on the inclusion criteria. Finally, no articles fulfill the criteria.

With the aim of mapping the relationship between psychosocial work environment and nurses' performance on studies that used the Copenhagen Psychosocial Questionnaire (COPSOQ), this review found no studies that met the eligibility criteria. As in nursing empty reviews are really rare, this review can be flagged as an "empty" one [29, 30], a fact that testifies the lack of research that studies the psychosocial component of nurses in its entire dimension.

The Copenhagen Psychosocial Questionnaire (COPSOQ) is a well-known and widely accepted means of measuring psychosocial working conditions in different occupational sectors. COPSOQ I, was originally developed in Denmark in 1997, recording a wide range of psychosocial working conditions [31]. In 2004/2005, a validation study was carried out in Denmark for the development of the second edition of the Copenhagen Psychosocial Questionnaire (COPSOQ II) [32]. Since 2013, an international study has been conducted by researchers from the COPSOQ network (www.copsoq-network.org) to develop the third edition of the Copenhagen Psychosocial Questionnaire (COPSOQ III) [33]. The purpose of COPSOQ is to improve and facilitate research and practical interventions in the workplace. COPSOQ is a multidimensional instrument that measures a wide range of psychological, social and work-related factors that affect health, vitality and mental health and are related to behavioral and cognitive stress[33-38].

The COPSOQ scale is divided into 22 subscales, the majority of which are completed on a 5-degree Likert scale (e.g., 1. Always, 2. Many times, 3. Sometimes, 4. A few times, 5. Never/almost never). The subscales are as follows: Quantitative requirements, cognitive requirements, emotional requirements, requirements that lead to the coverage of emotions, requirements related to the senses, influence at work, potential for development, degree of freedom at work, meaning at work, engagement with the work environment, predictability, clarity in roles, clash of roles, quality in headquarters, social support, support at work, social relations, group sentiment, job insecurity, satisfaction at work, general state of health, mental health, vibrancy, behavioral stress, physical stress and cognitive stress.

A total of 68 studies were found that used the COPSOQ questionnaire and none of them used it in full. However, the results of some of them are quite interesting. More specifically showed that nurses present high levels of psychosocial risks. In contrast to other health care workers, nurses present high emotional demands, low meaning of work, low commitment to the work place, low work pace, role conflict and low job satisfaction, increasing stress and burnout [39]. Also found that supervisors' support and managers leadership had an important influence on reducing their intention to leave [40, 41].

LIMITATIONS

Regarding study limitations, although the time limit span was chosen to obtain all evidence, none of the studies fulfill it. In addition, the study could include studies in languages other than English, such as Spanish and German. Finally, it would be of particular interest to include surveys using part of the questionnaire, presenting how they approached psychosocial risks in nurses.

CONCLUSIONS

The purpose of this review was to answer two main questions: "Can COPSOQ screen completely psychosocial risks of nurses' work environment?" "Which of these dimensions affect more nurses' psychosocial world?". None of the articles found helped in this direction. It has been shown that COPSOQ, even though it can fully and to their full extent outline the psychosocial risks, has not been used to its full extent, except in a fragmentary way in the nursing profession. Contributing to this is the fact that it is a tool that, to serve its purpose, has a fairly large volume, making it difficult for the study population to complete it.

The review highlighted the need for further research using the entire COPSOQ questionnaire in order to fully study the psychosocial risks that nurses face in their work environment.

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CASE STUDY

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COEXISTENCE OF PHEOCHROMOCYTOMA AND HYPERCORTISOLEMIA. DIAGNOSTIC DIFFICULTIES

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ABSTRACT

Aim: Our study aimed to present diagnostic problems in the case of hypercortisolism, pheochromocytoma, hypertension, type 2 diabetes, and chronic kidney disease.

Materials and Methods: Description of a patient with resistant hypertension admitted to the Department of Endocrinology for hormonal diagnostics. The results of hormonal tests and imaging tests before the procedure were analyzed, and the patient's condition was checked after the procedure. The analysis was extended with a literature review, considering the diagnostic problems in the described case. Electronic databases were the primary way to search, mainly MEDLINE and PubMed. We described a case of a 61-year-old woman diagnosed with right adrenal pheochromocytoma, hypercortisolemia, chronic kidney disease, drug-resistant hypertension, type 2 diabetes, and hypercholesterolemia. During hospitalization in the Department of Endocrinology, i.a., imaging tests and tests to assess adrenal function were performed. During the diagnostic process, a decision was made to perform surgical treatment of the pheochromocytoma, resulting in clinical improvement of the patient's condition.

Conclusions: The described case presents diagnostic problems endocrinologists face in the coexistence of several diseases. Often, the diagnosis to make a final diagnosis is complicated, hindered by the patient's multi-morbidity, as well as by the medications taken. There are few studies analyzing the coexistence of the diseases as described by us and their impact on the results of diagnostic tests that would facilitate the diagnosis.

KEY WORDS: pheochromocytoma, diagnostic difficulties, Cushing's syndrome, hypercortisolemia, pseudo-Cushing's syndrome

INTRODUCTION

Arterial hypertension can be defined as refractory when the following three conditions are met:

- appropriate treatment regimen (angiotensin-converting enzyme inhibitors (ACEI) / angiotensin receptor blockers (ARB) + calcium channel blocker + thiazide/thiazide-like diuretic) in optimal or maximally tolerated doses does not reduce blood pressure <140 and/or <90 mmHg,
- home measurements or 24/7 recording show a lack of pressure control,
- lack of pseudo-resistance and secondary hypertension.

In secondary hypertension, some conditions may be resistant to treatment. Such conditions include chronic kidney disease, atherosclerotic renal artery stenosis, and primary hyperaldosteronism. Less common diseases include phaeochromocytoma or Cushing's syndrome. Moreover, drug-resistant hypertension may be accompanied by diabetes or atherosclerotic cardiovascular disease. The use of various drugs is also not indifferent to the value of blood pressure. Its increase may be caused by non-steroidal anti-inflammatory drugs, glucocorticosteroids, and oral contraceptives often used by patients [1].

PHEOCHROMOCYTOMA

Pheochromocytoma (PCC) is a tumor arising from the adrenal medulla's chromaffin cells. Symptoms such as paroxysmal increases in blood pressure, headache, palpitations, pale skin, muscle tremors, and anxiety are caused by excessive production and release of catecholamines (adrenaline, noradrenaline, less often other biogenic amines). Rarely these tumors are clinically silent and are discovered incidentally in imaging studies. In such situations, we talk about incidentaloma [2].

In the event of symptoms, especially in a paroxysmal manner, detailed diagnostics aimed at pheochromocytoma should be initiated. From laboratory tests, the most useful is the determination of the excretion of fractionated methoxycatecholamines in a 24-hour urine collection and the concentration of methoxycatecholamines in plasma by HPLC (high-performance liquid chromatography). Computed tomography is the imaging test of first choice to determine the tumor's location, and in some cases, magnetic resonance imaging is necessary. There are also additional imaging tests that facilitate diagnosis,such as PET scintigraphy with the use of ^{18}F-fluorodeoxyglucose, PET ^{68}Ga-DOTATATE (Gallium-labeled DOTA peptides binding to somatostatin receptors), or PET scintigraphy with MIBG (metaidobenzylguanidine) labeled with radioactive iodine (^{131}I). Finally, the diagnosis is confirmed by the result of histopathological examination.

The differential diagnosis should include primary hypertension, pseudo-pheochromocytoma [3], menopause,

diabetes, and changes in the central nervous system. These conditions have similar symptoms, and catecholamine and metabolite levels may also be elevated, but the values are usually lower than in phaeochromocytoma. Moreover, these tumors enlarge over time and cause a mass effect.

The treatment of choice is the surgical removal of the catecholamine-secreting tumor. The choice of the surgery method (open or laparoscopic) is adapted to the clinical picture and laboratory and imaging test results. The treatment must be radical, as untreated tumors may lead to death due to cardiovascular causes [4].

ACTH-INDEPENDENT CUSHING'S SYNDROME

Cushing's syndrome is a syndrome caused by excess glucocorticoids. It is divided into an exogenous drug-related syndrome and an endogenous one, which is divided into ACTH-dependent and ACTH-independent. ACTH-dependent syndrome is associated with excessive production of ACTH, which then stimulates the adrenal glands to secrete cortisol. In ACTH-independent Cushing's syndrome, excessive cortisol secretion occurs independently of ACTH. It may be secreted by either autonomous adrenal tumors or macronodular/micronodular adrenal hyperplasia.

Cushing's syndrome should be suspected in a patient who presents the following symptoms: red or red-cyanotic stretch marks, atrophy of the proximal muscles of the lower limbs and the shoulder girdle, plethora, moon-shaped face, and abdominal obesity. This diagnosis should also be consideredfor an atypical course of hypertension, diabetes, osteoporosis,or an adrenal tumor accidentally detected in an imaging examination. Basic diagnostic tests include:

- so-called short dexamethasone inhibition test (1 mg dexamethasone overnight inhibition test),
- determination of cortisol in a 24-hour urine collection,
- late evening determination of cortisol in blood serum or saliva,
- determination of serum ACTH concentration, CRH stimulation test,
- imaging tests (CT, MRI, iodocholesterol scintigraphy).

In the case of ACTH-independent Cushing's syndrome, the following can be found: no circadian rhythm of cortisol secretion, as well as increased urinary cortisol excretion during the day, low plasma ACTH concentration, and no suppression of cortisol secretion in the short and long dexamethasone test.

In the case of an autonomous tumor of the adrenal cortex, the treatment of choice is its surgical removal after prior preparation of the patient with a steroidogenesis inhibitor. However, in the case of micronodular or macronodular hyperplasia of the adrenal glands, the removal of both adrenal glands is the treatment of choice [5].

PSEUDO-CUSHING (PC) SYNDROME

Common disorders such as obesity, poorly controlled diabetes, polycystic ovary syndrome, or alcoholism may activate the hypothalamic-pituitary-adrenal (HPA) axis and cause pseudo-Cushing syndrome. It is worth noting that the above-mentioned are more common in the population than Cushing's syndrome. In the case of obesity, research studies point to increased activation of enzymes involved in cortisol metabolism as the cause of hypercortisolemia. It is about, among others, the microsomal enzyme 11β-HSD. It is present in various tissues, e.g., in visceral fat, and is responsible for converting inactive cortisone into active cortisol.

Animal studies have shown that alcohol, by stimulating the paraventricular nucleus, increases the concentration of CRH by activating the HPA axis. On the other hand, liver failure, often co-occurring with alcoholism, impairs cortisol metabolism and consequently increases its concentration in the body. Pseudo-Cushing syndrome diagnosis remains a major challenge for physicians. So far, no specific test has been selected to diagnose or differentiate PC syndrome from Cushing's syndrome. It is important to have a holistic picture of the patient, carefully collected history, and correct interpretation of test results [6].

CASE REPORT

A 60-year-old female was admitted to the endocrinology department to diagnose drug-resistant hypertension. She also had a history of type 2 diabetes and chronic kidney disease. The patient was suspected of having ACTH-independent hypercortisolism. Physically, she presented features of hypercortisolism (abdominal obesity WHR 0.92, stretch marks on the abdomen, "buffalo" neck, "moon-shaped face"). The patient was treated with an alpha-adrenoceptor antagonist (4 mg) in combination with a beta-blocker (nebivolol). In addition, she was also taking a combination preparation: a thiazide-like diuretic, an angiotensin-converting enzyme inhibitor, a calcium channel blocker (indipamide, perindopril, amlodipine), and a potassium-sparing diuretic (spironolactone).

Due to diabetes, the patient took insulin (38 units daily) and metformin. In addition, due to hyperuricemia, the patient was taking allopurinol (100 mg).

Abdominal CT with contrast revealed a lesion in the right adrenal gland measuring 40x35x45 mm and having a density of 35 HU. CT lesions can be divided according to their density: low (<10 HU) may indicate an adenoma, while high (>10 HU, usually >30 HU) suggests adrenal carcinoma, pheochromocytoma or metastatic disease (usually kidney cancer, lung cancer) (Table 1). Contrast-enhanced abdominal MRI revealed a 38x35 mm structure in the right adrenal gland with features that excluded a lipid-rich adenoma. The radiologist suspected a pheochromocytoma or lesion of another character.

Tests assessing adrenal cortex and medulla function were performed during the intake of the patient's medications (Table 2). Very high daily excretion of methoxycatecholamines in the urine was documented twice: metanephrine 9391.15 µg/24h and 6202.94 µg/24h (with the norm up to 197 µg/24h) and normetanephrine 5398.11 and 3543.16 µg/24h (the norm up to 354 µg /24h). On the other hand, the daily excretion of 3-methoxytyramine was within normal limits in two measurements. The level of aldosterone, in the measurement made after resting in the supine position, was slightly elevated, 179.84 pg/ml (normal to 156.47 pg/ml).

Table 1. Differentiation of adrenal tumors based on the CT image [15].

Feature	Benign tumor	Malignant tumor	Metastasis
Size	<3-4 cm	>4 cm	variable, often <3 cm
Shape	oval/round	irregular	oval or irregular
Structure	homogeneous	heterogeneous, foci of necrosis, calcifications	heterogeneous, with different densities
Borders	irregular, sharp	irregular, unsharp	out of focus
Location	one-sided	one-sided	often bilateral
Density	<10 j.H.	>10 j.H. (usually >20 j.H.)	>10 j.H. (usually >20 j.H.)

Table 2. The results of laboratory tests in the patient during hospitalization before the surgical removal of the right adrenal gland.

Electrolytes					
Na+ [mmol\l]	134	140	136	136	
K+ [mmol\l]	4,6	4,7	5,4	4,8	
CL- [mmol\l]	100	104	105	106	
Ca [mmol\l]	2,35				
Creatinine [umol/l (n. 50,4-98,1)]					
	103,3	108,4	113,5	165,8	109,2
Diabetes assessment					
glucose [mmol/l (n. 3,8-5,5)]	8,21				
HbA1c [% (n.>5,7)]	9,2				
Assessment of pituitary function					
PRL [ng/ml (n. 3,46-19,4)]	4,27				
TSH [uIU/ml (n. 0,35-4,94)]	0,597				
FSH [mlU/ml (n. 0,95-11,95)]	47,45				
LH [mlU/ml (n. 0,57-12,07)]	24,94				
ACTH [pg/ml (n. 4,7-48,8)]	19,67				
Assessment of adrenal function					
ACTH 6:00 [pg/ml (n. 4,70-48,8)]	19,67				
ACTH 22:00 [pg/ml]	5,61				
cortisol 6:00 [ug/dl (n.3,7-19,4)]	16,8				
cortisol 22:00 [pg/dl]	7,6				
cortisol in a 24-hour urine collection [ug/24h (n. 4,3-176)]	18	33			
DHEAS [ug/dl (n. <246)]	148,8				

normetanephrine in a 24-hour urine collection by HPLC [ug/24h (n. 105-354)]	5398,11	3543,16	
metanephrine in a 24-hour urine collection by HPLC [ug/24h (n. 74-297)]	9397,15	6206,94	
3-methoxytyramine in a 24-hour urine collection by HPLC[ug/24h (n. 94-400)]	323,64	258,44	
Evaluation of the function of the renin angiotensin aldosterone system			
aldosterone after lying down [pg/ml (n. 14,21-156,47)]	179,84		
The long dexamethasone test			
	I day	III day	V day
cortisol [ug/dl]	16,8	4,8	15
ACTH [pg/ml]	26,65	6,17	27,7
cortisol in a 24-hour urine collection [ug/24h]		18	33

Plasma renin activity (PRA) has not been measured. A long dexamethasone suppression test confirmed non-ACTH-independent hypercortisolism. During hospitalization, renal failure worsened. The creatinine concentration was 165.8 µmol/l with a normal value of 98.1 µmol/l. After initiating hydration and treatment modification, improvement was achieved, and creatinine concentration decreased to 109.2 µmol/l. The patient was discharged, and PET-FDG (to exclude a malignant process) and surgery were recommended. Due to the COVID-19 pandemic, the date of the PET-FDG examination was distant, so a decision was made to perform the surgery before the imaging test.

Classical adrenalectomy of the right adrenal gland was performed, and the result of histopathological examination confirmed the diagnosis of phaeochromocytoma (PASS 2 points). The PASS scale (Pheochromocytoma of the Adrenal Gland Scales Score) takes into account the microscopic features of the tumor, e.g., vascular invasion, tumor capsule invasion, presence of atypical mitoses, the appearance of necrosis, and increased cellular pleomorphism. The maximum number of points that can be obtained on this scale is 20 points. A PASS of less than 4 points is characteristic for benign tumors, and at least 4 points for potentially malignant tumors. However, this scale is indicative and does not allow us to clearly determine whether the lesion is benign or malignant [7, 8]. After the operation, an improvement in blood pressure was obtained, which allowed discontinuation of all antihypertensive drugs previously taken. Captopril in a dose of 25mg was prescribed on an ad hoc basis. In the Holter examination, the mean blood pressure was 131/81 mmHg during the day and 121/69 mmHg at night. Also, the level of glycemia normalized - insulin was discontinued, and metformin was left in treatment.

Hormonal tests showed normalization of the circadian rhythm of ACTH and cortisol secretion, and hypercortisolemia was excluded in the dexamethasone suppression test (Table 3). In the 24-hour urine collection, a slightly increased excretion of normetanephrine was found - 492 µg/24 h (normal up to 354 µg/24 h). The result of the abdominal CT with contrast indicated the radical nature of the procedure.

DISCUSSION

The above example of a patient describes a situation that endocrinologists often must deal with. Abdominal CT scans revealed an adrenal tumor that met the criteria for a pheochromocytoma. However, the cause of hypercortisolemia was still unknown. The patient suffered from uncontrolled diabetes, which may have contributed to the activation of the hypothalamic-pituitary-adrenal axis and the development of pseudo-Cushing syndrome. Therefore, it was decided to conduct a long dexamethasone inhibition test, which indicated abnormalities in the functioning of the adrenal axis. Suspicion of a phaeochromocytoma is a contraindication to the above-mentioned examination. However, due to the patient's drug-resistant hypertension, it was necessary to perform it. When conducting the dexamethasone inhibition test, it is necessary to consider the glucocorticosteroid preparations and drugs that may interfere with the metabolism of dexamethasone (affecting the CYP3A4 cytochrome). These include beta-blockers, calcium channel blockers (amlodipine), and statins, which were used by the patient [9]. Due to the risk of a hypertensive crisis, weaning them was impossible. Valassi E. et al. conducted a study on 101 patients with Cushing's syndrome and PC syndrome, who were divided into groups: patients taking medications and patients not taking any medications. This study aimed to evaluate the specificity of the Dex-CRH (dexamethasone-corticoliberin) combined test and to evaluate the potential effects of concomitant medications. In the group of people with the PC syndrome not taking drugs, lower cortisol levels were shown after performing the combined dexamethasone suppression test compared to those taking drugs. The test's

Table 3. Results of laboratory tests in a patient during hospitalization after surgical removal of the right adrenal gland.

Electrolytes			
Na+ [mmol\l]	140		
K+ [mmol\l]	4,7		
CL- [mmol\l]	105		
Ca [mmol\l]	2,4		
Pi [mmol/l]	1,47		
Creatinine [umol/l (n. 50,4-98,1)]			
	93,6		
Diabetes assessment			
glucose [mmol/l (n. 3,8-5,5)]	6,04		
HbA1c [% (n.>5,7)]	9,6		
Assessment of adrenal function			
ACTH 6:00 [pg/ml (n. 4,70-48,8)]	11,56		
ACTH 22:00 [pg/ml]	7,17		
cortisol 6:00 [ug/dl (n.3,7-19,4)]	9,7		
cortisol 22:00 [pg/dl]	2,8		
cortisol in 24-hour urine collection [ug/24h (n. 4,3-176)]	22		
DHEAS [ug/dl (n. <246)]	113,8	136,4	
normetanephrine in 24-hour urine collection met.HPLC [ug/24h (n. 105-354)]	492,49		
metanephrine in 24-hour urine collection met.HPLC [ug/24h (n. 74-297)]	61,46		
3-methoxytyramine in 24-hour urine collection met.HPLC [ug/24h (n. 94-400)]	133,8		
Evaluation of the function of the renin angiotensin aldosterone system			
PRA after verticalization (2h) [ng/ml/h (n. 0,48-4,0)]	2,75		
aldosterone after verticalization (2h) [pg/ml (n. 13,37-233,55)]	105,39		
aldosterone [ng/dl] / PRA after verticalization (2h)	3,63		
Long test with dexamethasone			
	I day	III day	V day
cortisol [ug/dl]	12,8	<1,0	
ACTH [pg/ml]	40,33	<1,6	
cortisol in a 24-hour urine collection [ug/24h]	22	32,0	

specificity and sensitivity were significantly higher in the drug-free group. At the same time, the effect of multidrug therapy on the increase in the activity of the hypothalamic-pituitary-adrenal axis was demonstrated, which causes an increase in the resistance of this axis to equivalent doses of dexamethasone. No such relationship was found in people with Cushing's syndrome [9].

In endocrinology, a frequently used diagnostic tool is the study of the concentration of substances in body fluids. In the described case, it was crucial to test the concentration of substances in urine (methoxycatecholamines and cortisol) and blood (cortisol, ACTH). However, one should remember about the influence of various diseases and drugs on diuresis (water balance) and indirectly on the concentration of certain substances. Particular attention should be given to patients whose renal function is impaired. In the case of chronic renal failure (CKD), endocrinological diagnosis, e.g., Cushing's syndrome, is difficult due to changes in the HPA axis. This is due to the function of the kidneys – they are responsible for converting cortisol into inactive cortisone by steroid dehydrogenase. In CKD, the activity of this enzyme is reduced, which increases the half-life of cortisol [10]. There is a hypothesis that a good substitute for measuring the cortisol level in the blood is measuring the concentration of this hormone in saliva [11]. The cortisol concentration is higher than physiological, but the circadian rhythm and cortisol response to ACTH are maintained. In CKD patients, low doses of dexamethasone do not inhibit the axis, whereas higher doses do so [12]. Lynette K. et al., in their work, presented how GFR values affect the cortisol concentration in the urine. A significant difference was noted at GFR <60 ml/min when the cortisol concentration in the urine was significantly lower [13]. The same paper also highlighted the link between elevated cortisol levels and other conditions. It lists, among others, poorly controlled diabetes as a cause of HPA axis activation, which can cause hypercortisolemia and pseudo-Cushing syndrome. The patient had abnormal blood glucose and HbA1C levels. In addition, other studies show that uncontrolled type 2 diabetes increases the risk of Cushing's syndrome [14].

The patient's medical history analysis shows how complicated and difficult it can be to diagnose correctly. The final diagnosis is often made after the fact, as was the case here. After the removal of the right adrenal gland, the symptoms of hypercortisolemia subsided, which may indicate that the patient had pseudo-Cushing syndrome caused by excessive activation of the HPA axis. The result of the abdominal CT indicated the radicality of the procedure.

CONCLUSIONS

Despite the well-developed criteria for diagnosing specific disease entities, it is worth remembering about alternative diagnostic methods adapted to the clinical condition. It is important to select the appropriate test method, and one must pay attention to the specificity and sensitivity of the test.

The criteria for conducting some tests are very restrictive, and many patients (for various reasons) should be excluded from them. However, it should be remembered that each patient should be treated individually, and often the balance of benefits and risks speaks for conducting tests/procedures despite the presence of contraindications. Patients often take medications that can affect test results. Based on the above example, it can be concluded that metabolic disorders and drugs related to them are a group that particularly affects the test results.

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SHORT COMMUNICATION

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A NEW HYPOTHESIS IN THE TREATMENT OF RECURRENT GLIOBLASTOMA MULTIFORME (GBM). PART 1: INTRODUCTION

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ABSTRACT

Modern treatment of glioblastoma multiforme (GBM) is based on neurosurgical methods combined with radiotherapy and chemotherapy. The prognosis for patients with GBM is extremely poor. Often, complete removal of the tumor is impossible and it often recurs. Therefore, in addition to standard regimens, modern methods such as modulated electrohyperthermia, monoclonal antibodies and individualised multimodal immunotherapy (IMI) based on vaccines and oncolytic viruses are also used in the treatment of GBM. Radioiodine therapy (RIT) also holds out hope for an effective treatment of this extremely aggressive brain tumor. The expression of the sodium iodide symporter (NIS) gene has been proven to have a positive effect on the treatment of selected cancers. Research confirm the presence of expression of this gene in GBM cells, although only in animal studies. Is it possible and therapeutically effective to treat GBM with RIT without the use of an exogenous NIS gene? The safety of therapy is relevant, as the only more serious adverse effect may be hypothyroidism. The use of RIT requires further clinical studies in patients. Perhaps it is worth revolutionizing GBM therapy to give sufferers a "new life".

KEY WORDS: glioblastoma multiforme, sodium iodide symporter (NIS), radioiodine therapy (RIT), tumor, oncology

Contemporary treatment methods for glioblastoma multiforme (GBM) involve neurosurgical techniques combined with radiotherapy and chemotherapy. Considering the findings of previous laboratory studies, which indicate that the expression of the sodium iodide symporter (NIS) gene has a positive impact on the treatment of certain cancers, the following question arises: Is it possible to treat GBM solely with radioiodine therapy (RIT)? It is highly probable that NIS exhibits activity in GBM cells. Therefore, it seems that administering RIT through NIS could inhibit the progression of GBM. Who knows, then, whether NIS will act favorably through RIT and impede the progression of GBM? The sodium iodide symporter, also known as the NIS protein, is responsible for the uptake of iodide ions into the cell and plays a fundamental role in thyroid gland function. Previous studies on the sodium iodide symporter include, among others, demonstrating the expression of the NIS protein in thyrocytes and tissues of other organs, investigating the antigenicity of the symporter in autoimmune thyroid diseases, and uncovering the genetic factors influencing the diverse activity of the NIS protein. Notably, the expression of NIS has been detected not only in the thyroid but particularly in various tumor cells [1-6]. Hence, the question arises whether it is possible and therapeutically effective to utilize the NIS pathway for targeted GBM treatment.

The administration of RIT is entirely safe, with the only potential complication being thyroid dysfunction depending on the dosage. Indeed, recent studies have demonstrated that, for thyroid cancer, the maximum RIT dose is 37,000 MBq (1,000 mCi) [7-8]. This raises the question of whether it is worth considering the use of ablative RIT at a dosage of 740 MBq (20 mCi) for GBM in an outpatient setting with the possibility of repeat administration.

What do we have to lose? After all, patients with GBM have a very short lifespan, and the prognosis is exceptionally unfavorable. However, the observation of NIS gene expression has been identified in GBM, albeit only in animal laboratory studies [9]. Perhaps it is worth starting to practice and confirming the effectiveness of RIT in GBM therapy, as suggested by numerous scientific indicators.

As early as 1955, Amyes et al. localized brain tumors using radioactive iodine and phosphorus [9]. In this procedure, a needle probe was first employed, proving to be highly useful in swiftly locating and determining the affected area of the brain tumor.

Radioisotopes of various elements are increasingly being used in nuclear medicine. Herein lies the potential efficacy of beta radiation, such as RIT, beyond its imaging capabilities. Currently, the experimental NIS gene is being employed,

[*] This paper was written by members and invitees of the International Head and Neck Scientific Group (www.IHNSG.com)

but due to time constraints, it may be worth considering the prompt administration of RIT in GBM, at doses ranging from 740 MBq (20 mCi) to as high as 5550 MBq (150 mCi). It has been demonstrated that, among other applications, RIT accumulates up to 37,000 MBq (1,000 mCi) in thyroid tumors [10-11]. This further substantiates the notion that the use of RIT does not have a negative impact on healthy cells in the body; it may only result in thyroid dysfunction, as mentioned before. It is also worth noting that the radioactive iodine activity utilized in the treatment of multinodular toxic goiter is approximately 150-200 μCi/g of thyroid tissue, calculated using the formula: thyroid mass (g) x 150-200 μCi x 1/T24 iodine uptake at 24 hours. RIT can be administered in higher doses - ranging from 370 MBq (10 mCi) to 740 MBq (20 mCi) - in outpatient settings for conditions such as hyperthyroidism in Graves' disease or toxic multinodular goiter [12].

Complete removal of Grade IV GBM is not always feasible. Therefore, in accordance with the current motivation, in addition to the aforementioned standard therapies, namely neurosurgery [13], conventional radiotherapy [14], chemotherapy (e.g., temozolomide), modulated electro-hyperthermia (mEHT) [15-16], and administration of humanized monoclonal antibodies (e.g., pembrolizumab), the use of RIT [17-19] can be considered. Attention has also been drawn to targeted therapy utilizing tyrosine kinase inhibitors (imatinib, sunitinib, and sorafenib), as well as the application of novel drugs such as crizotinib, entrectinib, or larotrectinib [20-23]. NanoTherm® therapy is employed in patients with GBM who have exhausted conventional treatment methods [24]. Recently, personalized multimodal immunotherapy (IMI) based on anti-cancer vaccines [25-31] and oncolytic viruses [12, 32-33] has been developed.

It may be worthwhile to make efforts today and attempt to revolutionize the existing therapy through RIT, even without the genetic aspects of NIS, but in its classical form - just as before. What is extremely significant is that this therapy can be entirely cost-free, and even if there are charges involved, they do not have to be exorbitant. The only consequence would be thyroid dysfunction, which is merely a "complication." However, could we potentially gain a "new lease on life" as patients are liberated from GBM?

Similarly, RIT has been and continues to be used, drawing inspiration from the publications of Hermida et al. and Gursoy et al., in patients with amiodarone-induced thyrotoxicosis (AIT) with very low radioiodine uptake (RAIU) [34-37]. However, the authors of the study utilized very high activities of radioactive iodine (up to 2,960 MBq [80 mCi]), which are not routinely employed in the treatment of hyperthyroidism. No severe adverse effects were observed in patients following RIT therapy for AIT. They only experienced thyroid dysfunction [34-35]. In the course of AIT treatment, when antithyroid drugs (ATDs), including thionamide derivatives such as propylthiouracil (PTU) and imidazoles (MMI, tiamazole, Metizol), led to agranulocytosis, hepatitis, vasculitis, or lupus-like syndrome, the use of RIT became necessary [38-40].

Again, the authors of this discussion suggest that the use of RIT in personalized treatment for GBM can be an effective complement to other therapies, just as the use of RIT in AIT [34-35, 40]. This therapy can play a crucial role in cases of GBM recurrence. However, current clinical studies in humans are lacking, although positive results have been achieved in Wistar rats and mice through the combination of NIS gene therapy and RIT [41].

The application of RIT in patients with recurrent GBM appears to be a promising therapeutic option. The authors speculate that this will provide a completely new perspective within the treatment paradiGBM.

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CONFLICT OF INTEREST

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SHORT COMMUNICATION

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A NEW HYPOTHESIS IN THE TREATMENT OF RECURRENT GLIOBLASTOMA MULTIFORME (GBM). PART 2: IS THERE AN ALTERNATIVE THERAPY OPTION IN RECURRENT GBM WHEN ALL STANDARD TREATMENTS HAVE BEEN EXHAUSTED?

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ABSTRACT

Glioblastoma multiforme (GBM) is the most aggressive and malignant brain tumor. The average survival time for a patient diagnosed with GBM, using standard treatment methods, is several months. Besides the routinely applied treatments such as neurosurgery, radiotherapy, and chemotherapy, progress is being made in the field of oncology, offering hope for improved treatment outcomes. New treatment methods include individualized multimodal immunotherapy (IMI) and modulated electro-hyperthermia. The coauthor of the above series of articles (parts 1 and 2) - A.Cz. presents the concept of a new, potentially breakthrough treatment option for recurrent GBM. A.Cz. was diagnosed with GBM in August 2021. Exhaustion of standard treatment methods, as well as immunotherapy and virotherapy, only provided temporary relief. Unfortunately, after a few months, the disease recurred. Having little to lose, A.Cz. accepted an ablative dose of 2960 MBq (80 mCi) of I^{131}, based on available literature data. Three days before the administration of radioiodine therapy (RIT), A.Cz. prophylactically blocked the thyroid's ability to absorb the radioisotope. In June 2023, approximately 7 weeks after receiving single I^{131} dose, the MRI examination confirmed a 30% reduction in the tumor's size. Based on this, one can speculate that Iodine-131 therapy may be an alternative treatment option for GBM patients in the future. However, this hypothesis requires confirmation in further clinical studies.

KEY WORDS: glioblastoma multiforme, sodium iodide symporter (NIS), radioiodine therapy (RIT), I^{131}, tumor, oncology

Classical treatment of glioblastoma multiforme (GBM) as explained in previous article, includes neurosurgery [1], classical radiotherapy [2] and chemotherapy (e.g. temozolomide). At present, tyrosine kinase inhibitors (imatinib, sunitinib and sorafenib) are used [3-6]. Currently, the latest drugs are also used, such as crizotinib, entrectinib, larotrectinib or epirubicin [6-7]. NanoTherm® therapy is also used in patients with GBM (GBM IV stage), in whom conventional treatment methods have been exhausted [8]. Recently, individualised multimodal immunotherapy (IMI) has been developed based on cancer vaccines [9-11] and oncolytic viruses [12]. Abbas et al. [13] contributed to the development of cellular and molecular immunology.

We analyzed the possibility of treatment recurrent GBM with radioiodine therapy (RIT) alone, without using the sodium iodide symporter (NIS) gene. The NIS protein is found in about 20-30% of normal thyroid follicular cells and shows the diversity of its existence both within the thyroid follicles and within the thyrocytes of a given follicle [14-15]. This underestimated NIS is nothing more than a protein responsible for the active transport of iodine to the thyroid cell. Spitzweg et al. [16] discovered the presence of NIS in extrathyroid tissues and also in various tumours [17]. The functional NIS expression is the basis for the diagnostic and therapeutic use of radioactive iodine (RAI), which has been widely used in the treatment of differentiated thyroid cancer for 80 years [18].

One of the most important advantages of I^{131} as mentioned above is the fact that the administration is completely harmless, the only complication being hypothyroidism. Indeed, it has been shown recently that, for example, in the case of thyroid cancer, the maximum therapy is 37000 MBg (1000 mCi). Considering the NIS gene expression in animals with GBM, the question arises whether it is worth using RIT to treat GBM, but in a dose ranging from 740 MBq (20 mCi) [19-20] to as much as 5,550 MBq (150 mCi) [19, 21], for example?

Interestingly, more than 60 years ago, Amyeset et al. [24] determined the localization of brain tumors (in rats) with RAI and phosphorus. Whereas in 2006 and 2007 reported

[*] This paper was written by members and invitees of the International Head and Neck Scientific Group (www.IHNSG.com)

positive effects of experimental treatment of gliomas with RIT in animals – Wistar rats [22] and mice [23] – a combination of *gen*NIS and RIT genetics has been reported. In this time Amyeset et al. [24] also determined the location of brain tumours (in rats) using RAI and phosphorus.

At this point, it is essential to refer to the 2007 study by Mamelak and Jacoby [25] on synthetically produced scorpion venom (giant yellow Israeli scorpion venom). These researchers determined that GBM treatment with RAI and scorpion venom as a carrier, when targeted at glioma, had no negative effects on adjacent cells and organs. Clinical trials on a selected group of patients seem to confirm this. Phase II trials are currently underway to determine the effectiveness of dose multiplication. Mamelak and Jacoby [25] conducting research also led the phase I study. They determined that the key ingredient was TM-601, a synthetic version of a peptide or protein molecule found in the venom of the aforementioned Israeli scorpion. By binding to glioma cells, TM-601 can penetrate the blood-brain barrier, which allows access of most substances to the brain tissue from the bloodstream including RAI.

Phase II clinical trials are currently underway to determine efficacy dose multiplication. The same scientists report that if further studies confirm these latest findings, we may also be able to use it in GBM therapy: in combination with other methods of treatment, such as chemotherapy, as a synergistic effect may then occur. In other words, the ability of TM-601 to inhibit cancer growth may allow the dose of chemotherapy to be reduced to achieve the desired treatment effect.

Head author of this article A.Cz. has own experience with RIT. Being a nuclear medic, endocrinologist and internist, she has recently presented a proposal for the treatment of recurrent high-grade glioma with RAI, which is used in hyperthyroidism (toxic nodular goitre, Graves's Basedow, adenoma toxicum) [19] and malignant thyroid tumours [21]. Additionally, author A.Cz has been also diagnosed with GBM on August 2021. A tumour (grade IV glioma) was removed neurosurgery just after diagnosis. After surgery, she underwent radio- and chemotherapeutic treatment. Chemotherapy was then continued (five days a month at the Greater Poland Oncology Centre in Poznan). From November 2021 to April 2023, she started additional immunological (virotherapy) therapy and was given two doses of vaccination against GBM in Cologne, Germany, at IOZK (Immun-Onkologisches Zentrum Köln). During immunotherapy in IOZK, Keytruda (pembrolizumab) was introduced. On December, 2022, thanks to the painstaking and effective efforts of oncologists from WCPIT (Wielkopolskie Centrum Pulmonologii i Torakochirurgii/ Department of Oncology), Keytruda treatment was made available to A.Cz. under the National Health Fund. After controlled magnetic resonance imaging (MRI) on April 2023, while A.Cz. felt unwell, it turned out that there was a recurrence. An urgent consultation indicated the need for immediate chemo- and immunotherapy. While waiting for therapy, with little to lose, she took an ablative dose of 2960 MBq (80 mCi) of I^{131}. Three days before RIT, she blocked the iodine uptake of the thyroid prophylactically. On June 2023 – 7 weeks after taking one dose of RIT, the MRI confirmed a 30% reduction in tumor size.

Based on this result, we can speculate that RIT may be an alternative therapeutic option in patients with recurrent GBM, however, such a hypothesis requires further confirmation and verification in larger clinical trials.

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