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Pregnancy outcomes after assisted reproductive technology among women with endometriosis in Ukraine: results a multicenter study

Aidyn G. Salmanov[1,2], Volodymyr V. Artyomenko[3], Victor O. Rud[4], Olena A. Dyndar[5], Oleksandra Z. Dymarska[5], Svitlana M. Korniyenko[3], Orusia A. Kovalyshyn[6], Anastasia S. Padchenko[7], Vitalii S. Strakhovetskyi[8]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]INSTITUTE OF PEDIATRICS, OBSTETRICS AND GYNECOLOGY OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[3]ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE
[4]NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE
[5]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[6]LVIV MEDICAL INSTITUTE, LVIV, UKRAINE
[7]KYIV PERINATAL CENTER, KYIV, UKRAINE
[8]MEDICAL CENTRE "ASHERA", KHARKIV, UKRAINE

ABSTRACT

Aim: To evaluate the association between adverse pregnancy outcome, assisted reproductive technology (ART) and a previous diagnosis of endometriosis in Ukraine.

Materials and Methods: We conducted a multicentre retrospective cohort study was based on infertility surveillance data among women reproductive age from January 1st, 2017 to December 31st, 2021 in Ukraine. The patients from 10 Ukrainian regions who achieved singleton pregnancy by ART were included in this study. Linked hospital, pregnancy/birth and mortality data were used. Logistic regression analysis was performed to calculate odds ratios (OR) and 95 % confidence interval (CI) for the rates of adverse pregnancy outcomes.

Results: During study period within the cohort of 11,271 singleton births, 94 women with endometriosis diagnosed before birth delivered 102 infants. Compared with women without endometriosis, women with endometriosis had higher risks of preterm birth [adjusted odds ratio 1.33, 95% confidence interval (CI), 1.23-1.44]. Women with endometriosis had higher risks of antepartal bleeding/placental complications, pre-eclampsia and Caesarean section. There was no association between endometriosis and risk of SGA-birth or stillbirth.

Conclusions: Endometriosis and ART use are both independently associated with increased risk of preterm birth, antepartum haemorrhage, placenta praevia and planned birth. These findings are clinically relevant to obstetricians for distinguishing high- and low-risk pregnancies. Pregnant women with endometriosis require increased antenatal surveillance.

KEY WORDS: assisted reproduction technology, endometriosis, adverse pregnancy outcome, reproductive epidemiology, Ukraine

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INTRODUCTION

Women infertility is one of the most important complications in gynecology. The World Health Organization has identified infertility as a global public health problem. The global prevalence of infertility among females is reported to be 3.5% -16.7% [1]. The high prevalence of infertility worldwide, implies that the current assessment of the women reproductive system is far from perfect.

According to the literature, as a result of advances in technology and provision of services, an increasing number of infants are born as a result of assisted reproductive technology (ART) therapy. In developed countries, ART pregnancies represent 1.7% to 4.0% of all births [2], while 0.7% to 1.5% of all births in Ukraine are the result of ART. An estimate of at least 12 million births have resulted from assisted reproduction techniques, as announced by International Committee Monitoring Assisted Reproduction Technologies (ICMART) in the European Society of Human Reproduction and Embryology (ESHRE) meeting in 2023. Incorporating techniques such as intracytoplasmic sperm injection

(ICSI) and testicular sperm extraction (TESE), the rate of Assisted Reproduction Technique (ART) births is expected to surpass 4% of total births [3,4].

One of the causes of female infertility is a history of endometriosis [5]. Endometriosis is common gynecological disease in Ukraine, affecting 20% of reproductive age women and 50% of women seeking infertility evaluation. Recent epidemiological studies reported an association between endometriosis and adverse pregnancy outcomes. Some studies reported increased incidences of preterm birth, pregnancy-induced hypertension (PIH), and small for gestational age (SGA) babies in women with endometriosis, suggesting that endometriosis affects pregnancy outcomes [6,7].

Women with endometriosis are more likely to have difficulty conceiving and tend to receive infertility treatment, including assisted reproductive technology (ART) therapy, which in itself is a risk factor for preterm birth, PIH, and SGA babies [8,9]. However, concern is mounting over the safety of ART and its effect on maternal and fetal well-being. ART pregnancies have a significantly higher risk of multiple pregnancy and adverse perinatal outcomes, including preterm delivery, low birth weight, and birth defects. The most studies have suggested an increased risk of preeclampsia, gestational hypertension, placenta previa, and gestational diabetes in ART pregnancies [10, 11]. There is increasing evidence that pregnancies after Assisted Reproduction Techniques (ART) are associated with pre-term birth, low birthweight, congenital defects, and increased mortality rates [12].

Currently, no cohort study has yet examined the effects of endometriosis on pregnancy outcomes in pregnant Ukrainian women. Furthermore, it is unclear whether pregnancy outcomes in women with endometriosis are affected by ART. To our knowledge, only a few studies were conducted to study infertility in Ukraine within narrow clinical features [13, 14].

AIM

The aim of this study was to evaluate the association between adverse pregnancy outcome, ART and a previous diagnosis of endometriosis in Ukraine.

MATERIALS AND METHODS

DESIGN, SETTING AND STUDY POPULATION

We conducted a multicentre retrospective cohort study was based on infertility surveillance data among women reproductive age from January 1st, 2017 to December 31st, 2021 in Ukraine. We compiled list of the 15 Privat medical centers for family planning and reproductive health and 20 public maternity hospitals. Of these, only 12 medical centers and 14 maternity hospitals from 10 regions of Ukraine agreed to take part in our study. Participants: all female residents of Ukraine aged 19-45 years and their index singleton pregnancy of at least 20 weeks gestation or 400 g birthweight. The patients with endometriosis and without endometriosis who achieved singleton pregnancy by ART were included in this study. Exclusion criteria: complications before pregnancy including endometrial cancer, cervical cancer and post-canization.

DEFINITION

The definitions used in this study for the diagnosis of these complications was as follows. Antepartal haemorrhage/placental complications were defined as placental abruption, placenta praevia and other reasons for antepartal bleeding. Preterm birth was defined as delivery before 37 complete weeks of gestation. Placenta previa was defined as the condition in which the placenta at least partially covered the internal ostium. SGA defined as an infant weight less than the 10th percentile for the gestational age. Gestational age was determined on the basis of menstrual history, a prenatal examination, and ultrasound findings, such as gestational sac diameter, crown rump length, and biparietal diameter. ART includes information on vitro fertilization (IVF) treatment for the present pregnancy. Parity was categorized into nulliparous or parous women. Maternal age was defined as age in completed years at the time of delivery and was categorized as ≤20, 21–25, 26–30, 31–35 or 36 years and older. Women were categorized according to the Body mass index (BMI) as lean (BMI < 20.0 kg/m^2), normal (BMI 20.0–24.9), overweight (BMI 25.0–29.9) and obese (BMI of 30.0 or more). Women were categorized as non-smokers, moderate smokers (one to nine cigarettes per day), or heavy smokers (at least10 cigarettes per day).

DATA COLLECTION

In Ukraine, maternal characteristics are recorded in a standardized manner during a woman's first visit for antenatal care, which occurs before the 15th week of gestation in more than 95% of the pregnancies. Linked hospital, pregnancy/birth and mortality data were used. Infant outcomes were derived from birth data and included preterm birth. Data on endometriosis is based on diagnosis of discharge or out-patient visits at hospitals in Ukraine before delivery. The demographic data included the patient's age, parity, past medical

Table 1. Characteristics of women delivering singleton infants and univariate associations with risk of preterm birth in Ukraine, 2017-2021

Variable	Number of births (n= 11219)	Preterm birth (≤36 Weeks)		
		No. of cases (n= 560)	Rate per 100 births (%)	OR (95% CI)
Endometriosis				
Yes	102	7	6.86	1.39 (1.30–1.49)
No*	11,117	553	4.97	1.00
Age (year)				
≤20	225	15	6.66	1.28 (1.22–1.34)
21–25*	1,755	94	5.36	1.00
26–30	3,867	183	4.73	0.88 (0.86–0.90)
31–35	3,571	165	4.62	0.86 (0.84–0.88)
≥36	1,791	100	5.58	1.05 (1.02–1.07)
Data missing	10	2	–	
Parity				
0	4,833	299	6.19	1.54 (1.51–1.56)
≥1*	6,386	261	4.09	1.00
BMI (kg/m^2)				
≤19.9	982	53	5.39	1.22 (1.19–1.26)
20·0–24·9*	5,220	233	4.46	1.00
25·0–29·9	2,181	105	4.81	1.08 (1.06–1.10)
≥30·0	862	50	5.80	1.32 (1.29–1.36)
Data missing	1,974	119	–	–

Note:
OR, odds ratio; CI, confidence interval.
*The births with this characteristic served as the reference group. Values are based on live births.

history including surgical records, suspected causes of infertility, the number of transferred embryos and pregnancy outcomes. Data collection was performed by extracting data from medical records. We analyzed following adverse pregnancy outcomes: preterm birth, placenta previa, and SGA birth, stillbirth, Caesarean section, pre-eclampsia and antepartal haemorrhage. The women who had laparoscopically diagnosed endometriosis and women who were suspected to have endometriosis by transvaginal ultrasound or medical history but did not undergo laparoscopy were categorized as endometriosis. The other women who were not suspected to have endometriosis by transvaginal ultrasound or in whom the presence of endometriosis was denied by laparoscopy were categorized as denied presence of endometriosis. Gestational age was primarily based on prenatal ultrasound measurement if present or otherwise estimated on the recorded date of the first day of the last menstrual period. Information about stillbirth at 28 weeks of gestation or later, birthweight, and infant sex was obtained from the standardized paediatric record, routinely filled out immediately after delivery. The current study limited the analysis to women who delivered a singleton live birth or stillbirth at ≥22 weeks of gestation.

ETHICS

The study was approved by Shupyk National Healthcare University of Ukraine. The board did not require the women to provide informed consent. Ethical principles such as discretion and confidentiality, and beneficence and nonmaleficence to participants were strictly adhered to.

STATISTICAL ANALYSIS

All statistical analyses were performed using the SAS software (SAS Institute Inc., Cary, NC, USA). Fisher's exact test was used to calculate p values for the comparison of maternal characteristics with or without endometriosis. We used unconditional logistic-regression analysis to evaluate the association between diagnosis of endometriosis and preterm delivery and SGA birth. We also investigated the possible association between endometriosis and stillbirth, Caesarean section, pre-eclampsia

Table 2. Maternal characteristics associated with endometriosis among women delivering singleton infants in Ukraine, 2017-2021

Variable	Endometriosis			
	Yes		No	
	Number of births (n= 102)	Percentage of births, %	Number of births (n= 11,169)	Percentage of births, %
Age (year)				
≤20	–	–	227	2.03
21–25	6	5.88	1,757	15.73
26–30	25	24.5	3,856	34.52
31–35	40	39.2	3,547	31.75
≥36	31	30.39	1,771	15.85
Data missing	–	–	11	–
Parity				
0	47	46.07	4,815	43.11
≥1	55	53.93	6,354	56.89
BMI (kg/m^2)				
≤19.9	9	8.82	1,185	10.61
20.0–24.9	60	58.86	6,302	56.42
25.0–29.9	25	24.61	2,633	23.57
≥30.0	8	7.71	1,049	9.39

Note: BMI, Body mass index.

and antepartal bleeding. Odds ratios (OR), presented with 95% confidence intervals (CI) were calculated before and after adjustments for maternal characteristics. The estimates were adjusted for maternal age, parity, education, BMI, smoking and calendar year of birth of the child. In order to adjust for the effect of repeated pregnancies, estimates were calculated using the generalized estimating equation method with no major differences in the results. A *P* value of <0.05 was considered statistically significant.

RESULTS

During study period (2017-2021) within the cohort of 11,271 singleton births, 94 women with endometriosis diagnosed before birth delivered 102 infants. Among the 11,271 singleton births 37 cases of stillbirth (rate 3.28 per 1000 births). In this study the rate of preterm birth was higher among women with endometriosis compared with women without endometriosis. Women with low and high maternal age, with high or low BMI, and nulliparous women had higher rates of preterm birth. Characteristics of women delivering singleton infants and univariate associations with risk of preterm birth in Ukraine are presented in Table 1.

In this study compared with women without endometriosis, women with endometriosis were of higher maternal age and were more likely to be primiparous. There were no major differences in BMI and parity between women with and without endometriosis. Maternal characteristics associated with endometriosis in women giving birth to singleton children in Ukraine are shown in Table 2.

In the multivariable analysis we adjusted for confounders known to be associated with adverse pregnancy outcome, such as maternal age, parity and BMI. In the adjusted model, the OR for preterm birth was 1.33 (95% CI, 1.23–1.44). For preterm birth we also categorized deliveries into spontaneous and induced (Caesarean section) preterm birth. In present study the risk associated with endometriosis was higher for induced preterm birth, OR 1.61, 1.41–1.83, compared with spontaneous preterm birth, OR 1.22, 1.11–1.34. Crude and adjusted odds ratios (AORs) for adverse pregnancy outcome in women with and without endometriosis among singleton births in Ukraine are presented in Table 3.

In the multivariate analysis, endometriosis disease was not associated with risk for SGA birth or stillbirth. In this study as compared with women without endometriosis, women with endometriosis were at increased risk of pre-eclampsia. Among women with endometriosis the risk of antepartal bleeding including placental disorders was increased. Caesarean section was more common among women with endometriosis compared with women without endometriosis, and the risk was highest for prelabour Caesarean section. The use of ART was more prevalent in women with endometriosis compared with women without endometriosis (data not shown).

Table 3. Crude and adjusted odds ratios (AORs) for adverse pregnancy outcome in women with and without endometriosis among singleton births in Ukraine, 2017-2021

Adverse pregnancy outcome	Endometriosis (n=102)		No endometriosis (n=11,169)		Crude (95% CI)	Adjusted (95% CI)
	n	%	n	%		
Preterm Birth (≤36 Weeks)*	7	6.86	556	4.98	1.39 (1.30–1.49)	1.33 (1.23–1.44)
SGA-Birth	3	2.94	266	2.38	1.17 (1.05–1.30)	1.04 (0.92–1.17)
Stillbirth	1	0.98	36	0.32	1.23 (0.94–1.61)	1.02 (0.74–1.40)
Pre-eclampsia	4	3.92	322	2.88	1.17 (1.06–1.29)	1.13 (1.02–1.26)
Antepartal bleeding/placental complications	3	2.94	151	1.35	1.95 (1.75–2.18)	1.76 (1.56–1.99)
Caesarean section*	22	21.57	1,506	13.48	1.76 (1.69–1.84)	1.47 (1.40–1.54)

Note

SGA: small-for-gestational-age

Odds ratios have been adjusted for maternal age, BMI, parity, and year of birth of the child.

*For preterm birth and Caesarean section values are based on live births.

DISCUSSION

The results presented multicentre retrospective cohort study was based on infertility surveillance data among women reproductive age in Ukraine. In this study including 11,271 singleton births we assessed the association between adverse pregnancy outcome (preterm birth, SGA birth, stillbirth, Caesarean section, pre-eclampsia and antepartal haemorrhage), ART and a previous diagnosis of endometriosis in Ukraine. This study expands upon the previous reports [5, 11, 13, 14] and is the first study to publish the association between adverse pregnancy outcome, ART and a previous diagnosis of endometriosis in Ukraine. In the present study we found an increased risk of preterm delivery among women with endometriosis. We also found that women with endometriosis more frequently were affected by pre-eclampsia and antepartal haemorrhage. Moreover, delivery through Caesarean section was almost twice as common in this group as compared with women without endometriosis. In this study there was no association between endometriosis and risk of SGA-birth or stillbirth. The results from this study are accordance with previous studies [7, 15, 16].

ART pregnancy is known to carry a high risk of adverse pregnancy outcomes, compared with natural conception. However, the underlying mechanism of the increased risk for adverse outcomes in ART pregnancy remains unclear. Previous studies on obstetrical complications among women with endometriosis have demonstrated an increased risk of placental complications, leading to a higher risk of obstetrical hemorrhage in pregnancy and during labor. They also indicate a possible increased risk of preeclampsia, preterm birth (PTB) and low birthweight (LBW) [7,15-19]. Wennberg AL, et al. reported that the risk of placenta previa, cesarean delivery, PTB, and low LBW higher in ART than in spontaneous conception (SC) pregnancies for most maternal ages. In both ART pregnancies, the risk of hypertensive disorders in pregnancy (HDP), placenta previa, cesarean delivery, PTB, LBW, and SGA changed significantly with age. The AORs for adverse neonatal outcomes at advanced maternal age (>35 years) showed a greater increase in SC than in ART. The change in risk with age did not differ between ART for maternal outcomes at advanced maternal age [20]. However, these results have been inconsistent and often are based on small study populations. Also, the increased use of artificial reproductive techniques means that the women with endometriosis who become pregnant are different from populations studied earlier. Current consequences of endometriosis for pregnancy, birth and neonatal outcome therefore need to be clarified in a large contemporary cohort of women with endometriosis.

According to the literature, there are contradictory findings on the association between endometriosis and several adverse pregnancy outcomes, for example, some studies suggest increased risk of preterm birth, pregnancy hypertension and small-for-gestational age among women with endometriosis [7, 9]. However, many large studies including population-based cohorts have not considered the effect of ART use. This is important because women with endometriosis are more likely to have difficulty conceiving and are more likely to undergo treatment with ART, which has been shown to be associated with adverse pregnancy outcomes. Ibiebele I, et al. found increased risk of placenta praevia among women who used ART as well as among women who had endometriosis [15]. These

findings concur with studies among women who used ART that have consistently reported that those who also had endometriosis had increased risk of placenta praevia compared to those without endometriosis [16, 21]. The mechanisms underlying these observations are not entirely clear, although factors related to ART have been implicated. It has also been suggested that perturbed uterine peristalsis in women with endometriosis may influence the site of implantation and increase the risk of placenta praevia [22].

In this study we found endometriosis and ART use, separately and together, were independently associated with increased risk of antepartum haemorrhage. This finding was partially supported by Ibiebele I, et al., 2022 [15] and other studies that found increased odds of antepartum haemorrhage among women with endometriosis compared to those without endometriosis but further analysis among the subgroup of women who conceived using ART found no association between endometriosis and antepartum haemorrhage [23].

Our study found increased risk of planned birth (caesarean delivery or induction of labour) with both endometriosis and ART use independently and this may reflect the higher rates of placenta praevia in the endometriosis and ART groups. However, other studies did not find an association between endometriosis and induction of labour [23]. We found increased risk of preterm birth associated with endometriosis as well as ART use. This concurs with other cohort studies that found increased risk of preterm birth in women with endometriosis compared to women without endometriosis in both spontaneous and ART pregnancies [24]. Inflammation has been suggested as a possible pathway between endometriosis and preterm birth, while the underlying causes of infertility and ART procedures have been found to be associated with adverse pregnancy outcomes [15]. We observed an increased risk of pre-eclampsia among women with endometriosis. This finding is in contrast with other study, where pre-eclampsia surprisingly was found to be reduced in women with endometriosis [25]. However, several methodological aspects, including low response rate and possible selection bias may account for this result. In the present study, women with endometriosis generally had a lower BMI compared with women with no endometriosis, which is supported by other cohort study [26].

Our results suggest that women with endometriosis including those who used ART to achieve pregnancy are a higher-risk obstetric group requiring appropriate surveillance and management during their pregnancy. Against the background of the demographic crisis in Ukraine, which is characterized by a decrease in the birth rate and population depopulation, it is important to improve the reproductive health of the population, treat infertility, and help solve the problem of postponing the birth of children in wartime conditions. Improving the quality and accessibility of ART should become one of the priorities of the state's demographic policy.

STRENGTH AND LIMITATIONS

Our study has several strengths. To our knowledge, this is the first report in Ukraine to suggest the correlation of adverse pregnancy outcome, ART and a previous diagnosis of endometriosis in Ukraine. This study was only included ART pregnancies to avoid the influence of conception methods. This study expands upon the previous reports and is the first study to publish the association between adverse pregnancy outcome, ART and a previous diagnosis of endometriosis in Ukraine. No prior study on this issue is as large, up to date, or comprehensive in Ukraine. A limitation of this study was that we were not able to follow all women throughout their entire reproductive period. Therefore, the total number of deliveries and other pregnancy outcomes does not represent the full lifelong reproductive history. Some of the women with endometriosis were diagnosed solely based on clinical suspicion, some of which, therefore, could be misclassified. We were unable to assess the effect of endometriosis stage or typology on the study outcomes.

CONCLUSIONS

Pregnancies resulting from ART are associated with an increased risk of adverse perinatal outcomes compared with those following natural conception. We found that endometriosis to be a risk factor for preterm birth, irrespective of ART. Women with endometriosis may be more likely to be delivered by Caesarean section and to suffer from antepartal haemorrhage/placental complications and pre-eclampsia. These findings are clinically relevant to obstetricians for distinguishing high- and low-risk pregnancies. This information might be helpful for women and their providers when managing these pregnancies. Pregnant women with endometriosis require increased antenatal surveillance. Further studies are required to assess whether any modification is needed to conventional pregnancy monitoring for patients with endometriosis.

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CORRESPONDING AUTHOR
Aidyn G. Salmanov
Shupyk National Healthcare University of Ukraine,
9 Dorohozhytska St., 04112, Kyiv, Ukraine
e-mail: mozsago@gmail.com

ORCID AND CONTRIBUTIONSHIP
Aidyn G. Salmanov: 0000-0002-4673-1154 A C D E F
Volodymyr V. Artyomenko: 0000-0003-2490-375X B C D F
Victor O. Rud: 0000-0002-0768-6477 B C D F
Olena A. Dyndar: 0000-0002-0440-0410 B C D F
Oleksandra Z. Dymarska: 0009-0006-7275-9405 B C D F
Svitlana M. Korniyenko: 0000-0003-3743-426X B C D F
Orusia A. Kovalyshyn: 0000-0002-9710-0694 B C D F
Anastasia S. Padchenko: 0009-0007-6382-8955 B C D F
Vitalii S. Strakhovetskyi: 0000-0002-7528-1498 B C D F

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

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ORIGINAL ARTICLE

CONTENTS

Study of the influence of disease duration on glutatione-dependent ensymes dynamics in patients with paranoid schizophrenia

Ivan R. Romash, Iryna B. Romash, Mykhaylo M. Pustovoyt
IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: The objective of the research was to conduct a comprehensive longitudinal analysis of the temporal dynamics of glutathione system functionality in individuals diagnosed with paranoid schizophrenia. Specifically, the research was focused on investigating variations in the profiles of glutathione-dependent enzymes, with meticulous consideration given to the duration of the illness.

Materials and Methods: The study group comprised 300 individuals officially diagnosed with 'Paranoid Schizophrenia,' subdivided into five subgroups, each consisting of 60 patients. The subgroups were defined as follows: Subgroup I included 60 patients with a disease duration ranging from 3 to 5 years; Subgroup II comprised 60 patients with a duration of 6 to 10 years; Subgroup III consisted of 60 patients with a duration of 11 to 15 years; Subgroup IV included 60 patients with a duration of 16 to 20 years; and Subgroup V encompassed 60 patients with a duration of 21 years and older. The comparison group comprised 20 patients diagnosed with "Primary psychotic episode".

Results: The research demonstrates a consistent and noteworthy reduction in the enzymatic activities of glutathione peroxidase, glutathione reductase, and glutathione-S-transferase in various Subgroups of paranoid schizophrenia patients. The observed declines are particularly prominent within the first 3-5 years of the illness, show casing statistically significant reductions. Patients with prolonged illness durations, especially surpassing 21 years, display substantial reductions in all three enzymes, suggesting a cumulative enzymatic impact associated with prolonged illness.

Conclusions: The identification of critical periods of inhibition in the glutathione protection chain, provides valuable information about potential therapeutic interventions for individuals with paranoid schizophrenia.

KEY WORDS: schizophrenia, antioxidant defense system, oxidative stress, glutathione-dependent enzymes, neuroinflammation, neuroplasticity

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INTRODUCTION

The most prevalent contemporary mental illnesses include schizophrenia and schizotypal disorders. Schizophrenia is a severe mental illness and affects approximately 1% to 2.5% of the global population, according to various estimates [1-3]. Unfortunately, the lack of dependable biomarkers poses a formidable challenge to the early-stage detection of chizophrenia. Consequently, the development of effective early diagnosis and therapy for schizophrenia holds paramount significance in medicine, particularly within the field of psychiatry.

According to scientific data, oxidative stress (OS) becomes a convergence point for the influence of genetic, environmental and behavioral risk factors for schizophrenia during neuro-development. OS is caused by an excess of free radicals generated due to cellular metabolic stress and impairment of the antioxidant defense system (ADS), known to cause membrane dysfunction involved in the pathophysiology of schizophrenia. Although it cannot be the primary cause of schizophrenia, there is substantial evidence indicating that OS can complicate the course and cause difficulties in the treatment of patients within this category. Numerous scientific works indicate the complex dynamics of redox regulation mechanisms and their modulation in schizophrenia [1, 4].

The antioxidant system as a pivotal defense mechanism within the human body, assumes a fundamental role by actively participating in metabolic processes, thereby maintaining balance across all phases of free radical peroxidation (FRP) [5]. This intricate defense system orchestrates interconnected reverse redox reactions (RR), involving metal ions, glutathione, tocopherols, phospholipids, "trigger hormones", and other bioactive substances. Otherwise speaking, the human body is endowed with a precision-targeted system designed to protect it from oxidative damage induced by reactive oxidants. The glutathione system plays a key role in providing antioxidant protection.

This system comprises reduced glutathione (GSH) and enzymes of its metabolism, including glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) [6]. This intricate network of molecules and enzymes underscores the sophisticated and finely tuned nature of the body's defense against OS, contributing significantly to the maintenance of cellular homeostasis.

AIM

To conduct a comprehensive longitudinal analysis of the temporal dynamics of glutathione system functionality in individuals diagnosed with paranoid schizophrenia. Specifically, the research was focused on investigating variations in the profiles of glutathione-dependent enzymes, with meticulous consideration given to the duration of the illness. Through this investigation, the objective was to provide nuanced insights into the relation between disease progression and the complicated molecular alterations within the glutathione system, thereby contributing to a deeper understanding of the pathophysiological mechanisms underlying paranoid schizophrenia.

MATERIALS AND METHODS

The study involved the analysis of glutathione-dependent enzymes indicators in the blood serum of 320 patients in order to achieve the research objective. The study group comprised 300 individuals officially diagnosed with 'Paranoid Schizophrenia,' subdivided into five subgroups, each consisting of 60 patients. The criteria for such distribution were based on the duration of the primary disease, according to official medical documentation. The subgroups were defined as follows: Subgroup I included 60 patients with a disease duration ranging from 3 to 5 years; Subgroup II comprised 60 patients with a duration of 6 to 10 years; Subgroup III consisted of 60 patients with a duration of 11 to 15 years; Subgroup IV included 60 patients with a duration of 16 to 20 years; and Subgroup V encompassed 60 patients with a duration of 21 years and older. The comparison group comprised 20 patients diagnosed with "Primary psychotic episode". The clinical assessment of patients was performed by using the Positive and Negative Syndrome Scale (PANSS).

Blood plasma from study participants was collected at a fixed time, between 07:00 and 09:00, following an overnight fast. Subsequent to sample centrifugation, the plasma was transferred to 1.5 ml Eppendorf tubes and frozen at -30 °C. Biological markers were determined by a specialist from the accredited biochemical laboratory of the Center for Microelementology of the Ivano-Frankivsk National Medical University at the Department of Biological and Medical Chemistry named after Academician Babenko G.O. The analysis was conducted by standard methods and strictly adhered to established protocols.

The functional state of the enzymatic system of detoxification and the antioxidant defense system (ADS) was assessed through the activity levels of glutathione-dependent enzymes. Specifically, the activity of glutathione peroxidase was determined by the reaction of reduced glutathione with tert-Butyl hydroperoxide (tBuOOH) and calculated in micromoles of oxidized glutathione per milligram of protein per minute (μmol/(min×mg)). Glutathione reductase activity was measured by the rate of change in optical density at 340 nm due to the oxidation of Nicotinamide adenine dinucleotide phosphate (NADPH) and expressed in nmol/(min × ml). The activity of glutathione-S-transferase was determined by the rate of formation of glutathione-S-conjugates between reduced glutathione and 1-chloro-2,4-dinitrobenzene (CDNB), expressed in nmol/(min×mg).

The research adhered to ethical guidelines, including the Declaration of Helsinki (1964-2013), GCP (1996), ICH GCP (1996), the Council of Europe Convention on Human Rights and of Biomedicine (from 04.04.1997), EU Directive No. 609 (from 11.24.1986), and relevant Ukrainian Ministry of Health orders. Patient participation was possible upon signing the 'Voluntary Informed Consent of the Patient to Participate in the Study' form. The clinical and laboratory research protocol received approval from the Ivano-Frankivsk National Medical University Ethics Committee (protocol N 135/23 dated 05/24/2023).

The «STATISTICA 8.0» software package (StatSoft, Serial STA862D175437Q) and the statistical functions of «Microsoft Excel 2016» were used for the statistical analysis of the obtained results. The reliability of the data was confirmed by calculating errors for relative values. The probability of data differences between the compared groups was established using the t (Student) coefficient, with the accuracy of the error-free prediction determined according to the table. Quantitative characteristics were described using the arithmetic mean (M) and standard error (±m) [7].

RESULTS

According to Fig. 1, a probable decrease in the value of glutathione peroxidase was observed in all experimental subgroups. Subgroups I and II, including patients with a disease duration of 3-5 and 6-10 years, respec-

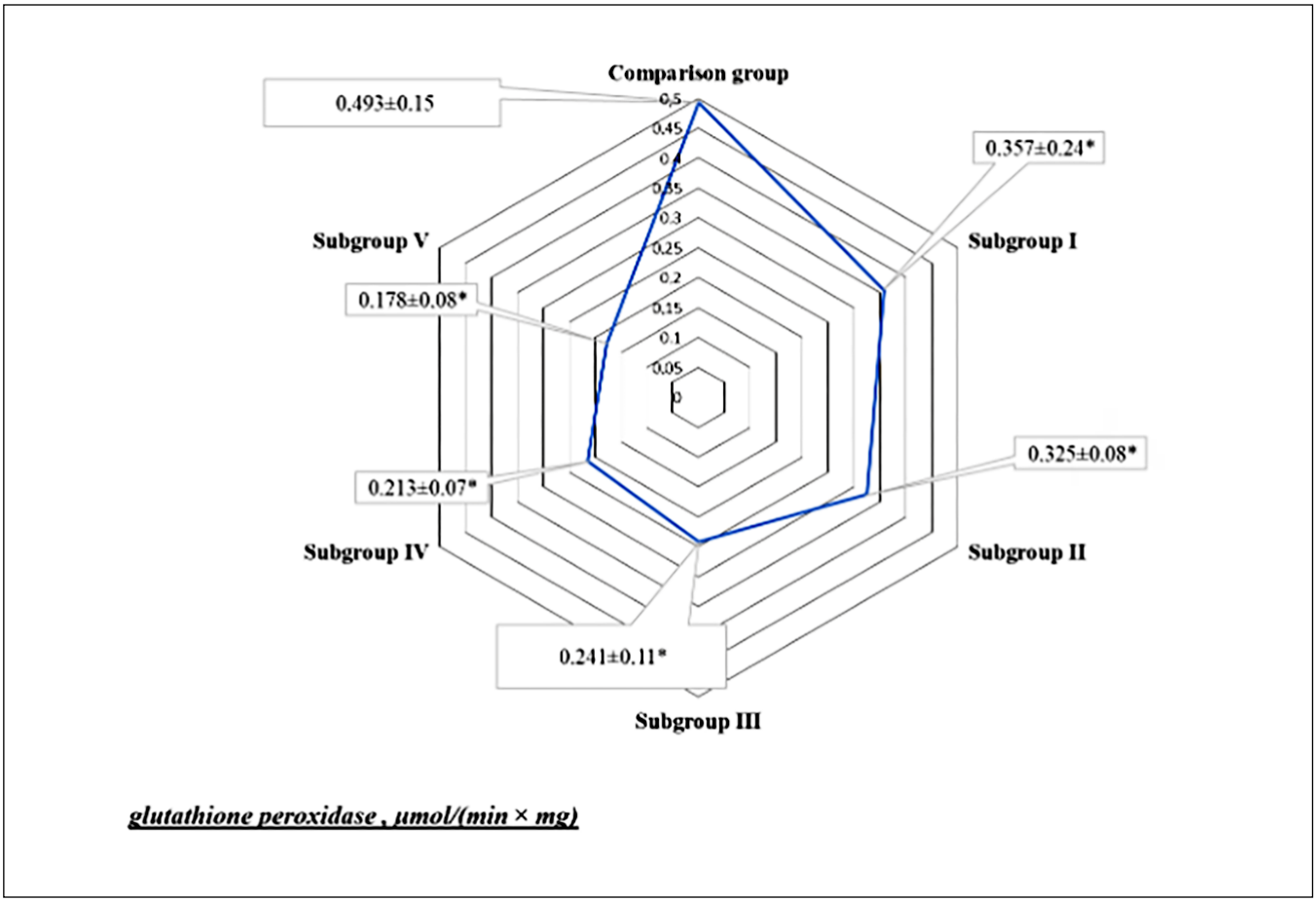

Fig. 1. Dynamics of the glutathione peroxidase indicator in patients with paranoid schizophrenia depending on the disease duration.
Note: * – ($p<0.05$) the data are reliable between the Comparison and Study Subgroups.

tively, demonstrated statistically significant reductions, emphasizing early changes in these enzymatic activities. This indicator was 0.357±0.24 μmol/(min×mg) in Subgroup I which was 27.5% less than in the Comparison group ($p<0.05$). In Subgroup II, this indicator constituted 0.325±0.08 μmol/(min×mg), representing 34.07% decrease compared to the Comparison group ($p<0.05$). In Subgroup III, the value of glutathione peroxidase was 0.241±0.11, more than 50% lower, respectively ($p<0.05$). A sharp decrease in the level of glutathione peroxidase by more than half was observed in patients who suffered from the paranoid form of schizophrenia for more than 10 years: by 51.11% in Subgroup III, by 56.79% in Subgroup IV, by 63.89% in Subgroup V.

Monitoring the dynamics of this indicator, we notice that the first "downfall" occured within the first 3-5 years of the disease, constituting a decrease of 27.58%. Over the next five years, this indicator decreased by another 6.49%. The second sharp "downfall" was observed after 10 years of the disease. The indicator decreased by more than 17% in the examined patients of Subgroup III compared to patients in Subgroup II. In patients of Subgroup IV, the history of the main disease was 16-20 years, and the value of glutathione peroxidase was 0.213±0.07 μmol/(min×mg), being 56.79% lower than in the Comparison group and 5.67% lower than in patients from Subgroup III. In Subgroup V patients suffering from paranoid schizophrenia over 21 years, this indicator was 0.178±0.08 μmol/(min×mg), which was 63.89% and 7.09% lower, respectively ($p<0.05$).

According to the data presented in Fig. 2, the glutathione reductase indicator was 0.089±0.002 nmol/(min×ml) in the patients of experimental Subgroup I, who were sick for 3-5 years. This was only 8.24% less than in the Comparison group ($p>0.05$). In Subgroup II, this indicator was 10.30% less than in the Comparison group and amounted to 0.073±0.004 nmol/(min×mg) (<0.05). In Subgroup III it was by 42.26% lower and constituted 0.056±0.007 nmol/(min×mg), respectively (<0.05). The glutathione reductase indicator was 0.038±0.005 nmol/(min × mg) in the patients of Subgroup IV, where the history of the main disease was 16-20 years. This was 60.82% less than in the Comparison group (<0.05). The indicator amounted 0.029±0.003 nmol/(min×mg), 70.10%, respectively (<0.05) in Subgroup V patients suffering from paranoid schizophrenia over 21 years.

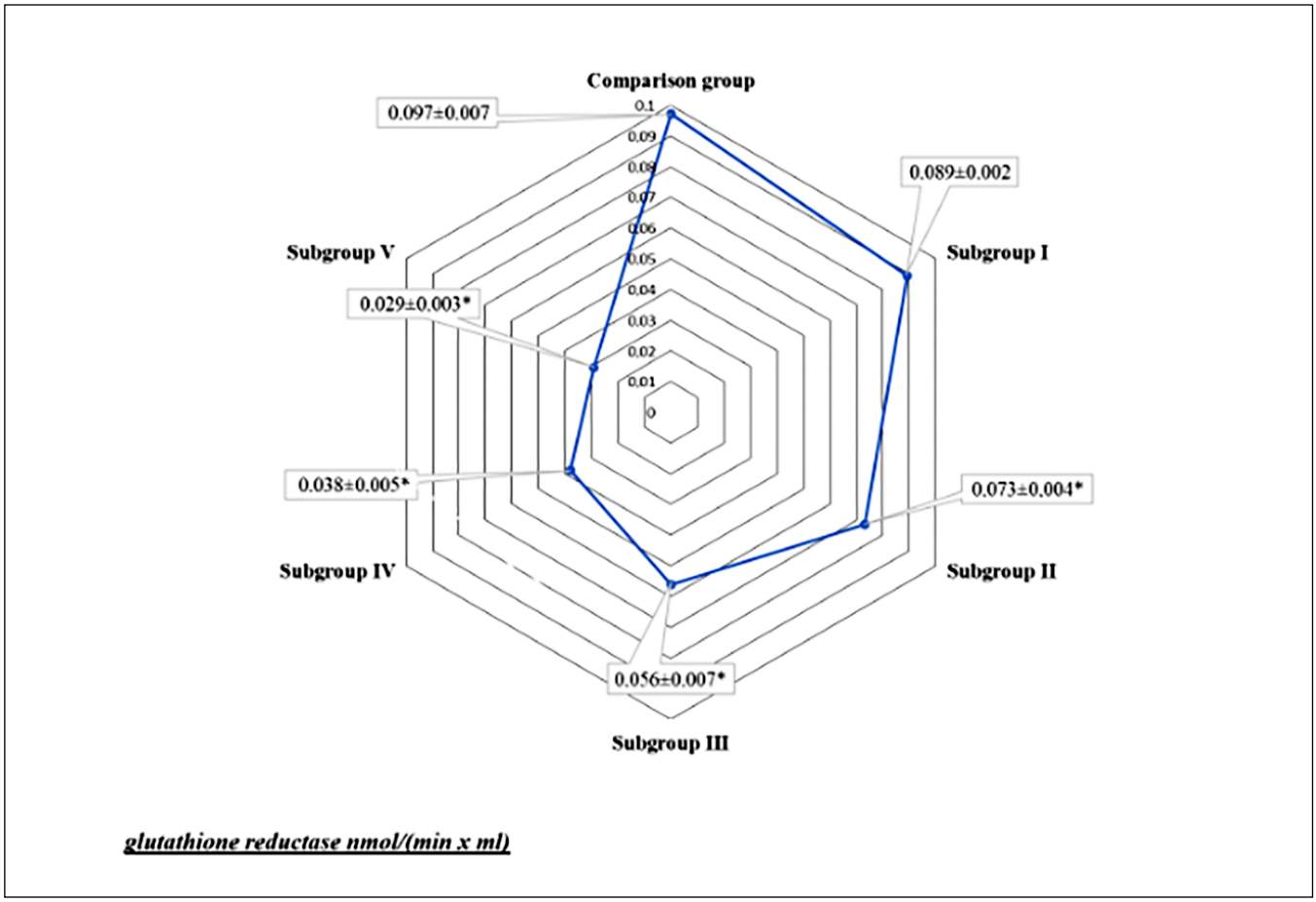

Fig. 2. Dynamics of the glutathione reductase indicator in patients with paranoid schizophrenia depending on the disease duration.
Note: * – ($p<0.05$) the data are reliable between the Comparison and Study Subgroups.

The obtained results, showing a decrease in the activity of the glutathione reductase enzyme, may suggest a physiological deficiency of reduced glutathione as a crucial component of the glutathione pool. Consequently, this deficiency could weaken the antioxidant defense system, leading to an increase in peroxide levels, the onset of oxidative stress, and potential tissue damage.

The study of the enzyme glutathione-S-transferase, responsible for the detoxification segment of the glutathione antioxidant system, also revealed a decrease in its activity. Similar to glutathione peroxidase and glutathione reductase, a consistent decrease in glutathione-S-transferase activity was observed in all subgroups, with significant decreases becoming apparent after 5 years of the disease (Fig.3). This indicator was 1.072±0.122 nmol/(min×mg) in Subgroup I which was 21.80% less than in the Comparison group ($p>0.05$). In Subgroup II, this indicator was 36.32% less than in the Comparison group and amounted to 0.873±0.162 nmol/(min × mg) (<0.05).). In Subgroup III, where the history of the main disease was from 11 to 15 years, it was 65.28% lower and constituted 0.476±0.090 nmol/(min×mg), respectively (<0.05). The glutathione-S-transferase index was 0.432±0.042 nmol/(min×mg) in patients of Subgroup IV who suffered from paranoid schizophrenia from 16 to 20 years, which was 68.49% lower, respectively (<0.05). This indicator was 0.324±0.075 nmol/(min×mg) in the patients of Subgroup V suffering from paranoid schizophrenia over 21 years, which was 76.36% less than in the Comparison group (<0.05). This significant decrease of enzyme, especially in Subgroups IV and V, reinforces the notion of cumulative enzymatic changes with prolonged illness duration.

DISCUSSION

The glutathione system functions cohesively, combining diverse biochemical detoxification mechanisms to preserve a state of normal redox homeostasis. Its components participate in both enzymatic (GPx, GR, GST) and non-enzymatic (GSH) reactions of antioxidant defense. The first ones are genetically programmed, specialized, and the most effective: GPx is responsible for the inactivation of free radicals, and GR, GST are responsible for the recovery of oxidized compounds.

The study of glutathione-dependent enzymes in patients with paranoid schizophrenia reveals interesting

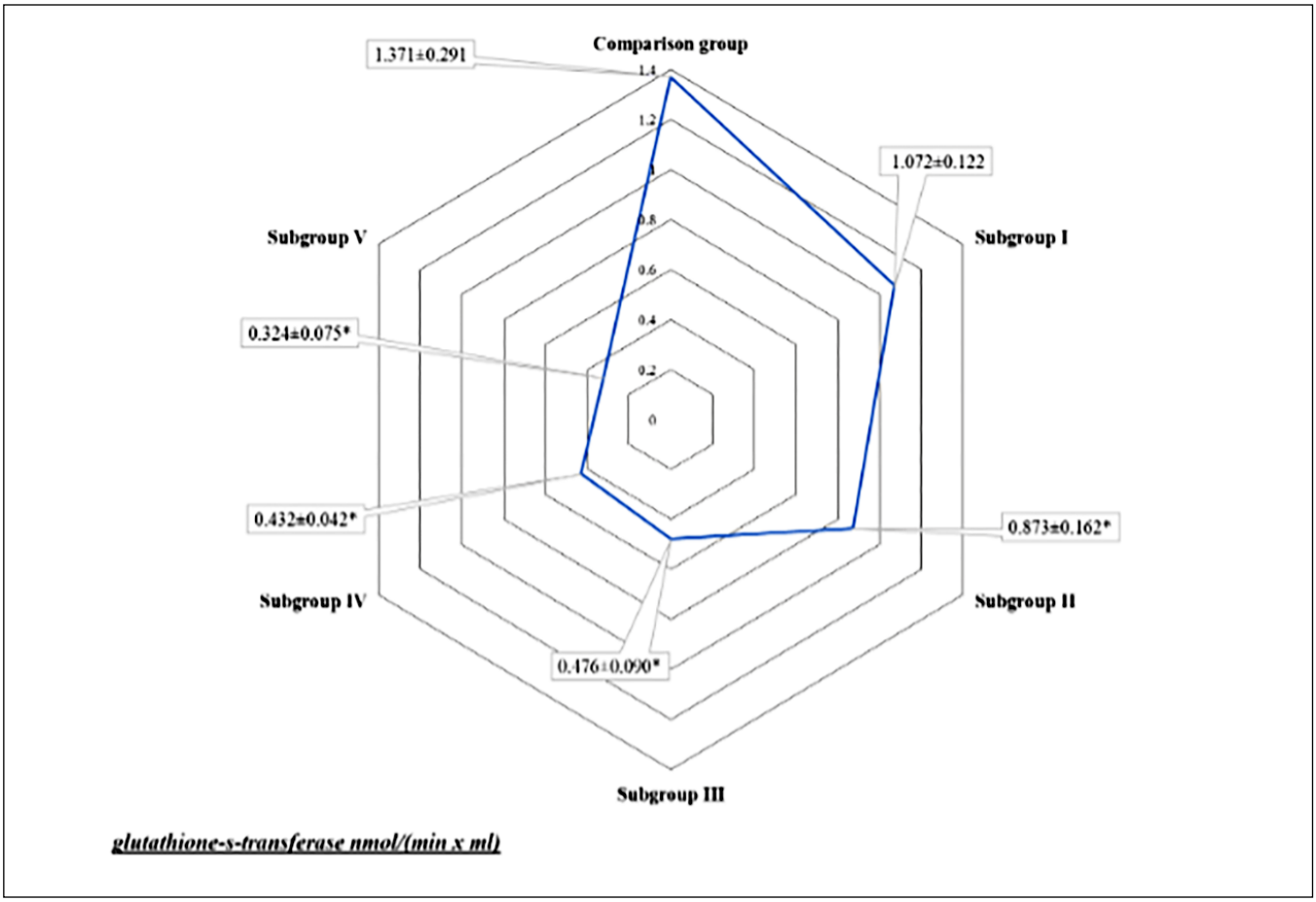

Fig. 3. Dynamics of the glutathione-S-transferase indicator in patients with paranoid schizophrenia depending on the disease duration.
Note: * – ($p<0.05$) the data are reliable between the Comparison and Study Subgroups.

aspects of changes in the enzymatic system during the long course of the disease. According to the analysis of the obtained data, the paranoid form of schizophrenia is accompanied by the development of insufficient functioning of the glutathione system components, and the dynamics of its indicators testifies to the exhaustion of antioxidant protection mechanisms, as a result of which the level of peroxide compounds significantly increases, and antioxidant homeostasis is disturbed

The data we obtained indicated a consistent decrease in the level of glutathione peroxidase in all experimental subgroups. Subgroups I and II, including patients with a disease duration of 3-5 and 6-10 years, respectively, showed a statistically significant decrease, highlighting the early changes in these enzymatic activities. It is noteworthy that the most dramatic decrease in the level of glutathione peroxidase was observed in the patients suffering for more than ten years, and a decrease of more than 50% was observed in subgroup III. This process was even more intensified in subgroups IV and V, demonstrating a significant decrease in the activity of this enzyme. The analysis revealed distinct periods of decline, highlighting potentially critical stages in the progression of paranoid schizophrenia. Our findings align with research conducted by Djordjević V. and colleagues. In their study, scientists observed a significant decrease in GPx activity in the patients who had more than one episode and in those experiencing schizophrenia for over a year. In addition, their results confirmed the existence of OS in the early years of clinically evident schizophrenia, with its correlation to the number of psychotic episodes and the illness duration [8].

In contrast to GPx, reduced levels of GR were evident mainly in patients with a long history of the disease. A significant decrease in its activity was observed in subgroups III, IV, and V. Similar to GPx and GR, a consistent decrease in GST activity was observed in all subgroups with a significant decrease becoming evident after 5 years of the disease. A pronounced decrease in the level of this enzyme, especially in subgroups IV and V, confirms the idea of cumulative enzymatic changes during the long course of the disease. In our opinion, the observed "failures" in enzyme levels indicated complex temporal dynamics emphasizing the need for long-term monitoring and intervention.

Our data partly contradict the findings of Goh, Xue Xin, and their co-authors. In their meta-analysis, includ-

ing studies published from 1964 to 2021, they sought the cause of the disturbance in the antioxidant defense system in case of schizophrenia. In their work, the scientists confirmed that patients with schizophrenia had disorders of the antioxidant defense system, but of the non-enzymatic antioxidant system. Notably, they observed that the antioxidant status was more compromised in drug-naive patients, implying that antipsychotics might enhance the antioxidant defense system [9].

Reactive oxygen species (ROS) are a collective term by their nature including not only radicals but also non-radical molecules of high reactivity, capable of triggering OS in a living organism. Under such conditions, a violation of coordinated metabolic reactions occurs: the formation of peroxides, aldehydes, and ketones. The effectiveness of their detoxification in the body depends on the functional fullness of the enzyme systems responsible for their biotransformation. The primary source of ROS has been scientifically proven to be mitochondria, organelles playing a key role in the energy supply of the cell. In particular, this is caused by a change in the permeability of mitochondrial membranes, namely the appearance of a specific complex of pores. According to the literature, even in a state of rest in the respiratory chain of mitochondria, ROS are formed as intermediate products, some of which are transformed entering the cellular space and start a chain of destructive transformations in biological structures.

OS also affects mitochondrial dysfunction and neuroinflammation [10]. It is well known that all types of neuroplastic processes are inhibited in the conditions of an active inflammatory process. All types of neuroplastic processes are known to be suppressed in conditions of an active inflammatory process. Their role is important in the pathogenesis of schizophrenia [11, 12].

The results of the conducted study emphasize the complexity of the glutathione system response to the progression of paranoid schizophrenia with clear enzyme-specific patterns and temporal dynamics. The observed decrease in enzyme activity indicates a potential relationship between disease duration and changes in the glutathione system, implicating oxidative stress in the pathophysiology of paranoid schizophrenia [9, 12, 13].

LIMITATIONS AND FUTURE DIRECTIONS

While this study provides valuable insights, it is essential to acknowledge certain limitations. The cross-sectional design limits the ability to establish causal relationships, emphasizing the need for longitudinal investigations.

The study only included a relatively small group of participants. Larger studies with longer follow-up periods are needed to confirm these findings.

The study did not explore the potential causes of decreased antioxidant enzyme activity. Investigating the underlying mechanisms could provide valuable insights into the pathogenesis of schizophrenia. In addition, external factors influencing enzyme activities should be considered in future research.

The focus was on paranoid schizophrenia; however, it would be interesting to compare the activity of glutathione-dependent enzymes to other subtypes of schizophrenia to see if there are any differences.

CONCLUSIONS

The identification of critical periods of inhibition in the glutathione protection chain, as found in this study, provides valuable information about potential therapeutic interventions for individuals with paranoid schizophrenia. Understanding these enzymatic changes is crucial for the development of targeted strategies that can eliminate the observed imbalances in the glutathione antioxidant system. The significance of reduced enzyme activity in the pathogenesis of paranoid schizophrenia highlights the need for more detailed investigations. Further research in this direction comprise the development of specific interventions that could enhance the overall treatment and management of paranoid schizophrenia, ultimately improving outcomes for affected individuals.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Ivan R. Romash
Ivano-Frankivsk national medical university
5/7 Pylyp Orlyk st., 76010 Ivano-Frankivsk, Ukraine
e-mail: iromashr@gmail.com

ORCID AND CONTRIBUTIONSHIP

Ivan R. Romash: 0000-0002-6591-6613 A B C D E F
Iryna B. Romash: 0000-0002-9749-778 C D E F
Mykhaylo M. Pustovoyt: 0000-0001-8689-6220 E F

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

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ORIGINAL ARTICLE

CONTENTS

Indicators of biochemical control of diabetes mellitus during limited availability of health service in the context of hypoglycemic therapy

Marcin Kleibert[1], Beata Mrozikiewicz-Rakowska[2], Klaudia Korycka[3], Iga Płachta[3], Patrycja Małgorzata Bąk[3], Daniel Bałut[3], Jakub Zieliński[4], Wojciech Zgliczyński[2]

[1]CHAIR AND DEPARTMENT OF EXPERIMENTAL AND CLINICAL PHYSIOLOGY, LABORATORY OF CENTER FOR PRECLINICAL RESEARCH, MEDICAL UNIVERSITY OF WARSAW, WARSAW, POLAND

[2]DEPARTMENT OF ENDOCRINOLOGY, CENTRE OF POSTGRADUATE MEDICAL EDUCATION, BIELANSKI HOSPITAL, WARSAW, POLAND

[3]FACULTY OF MEDICINE, MEDICAL UNIVERSITY OF WARSAW, WARSAW, POLAND

[4]INTERDISCIPLINARY CENTRE FOR MATHEMATICAL AND COMPUTATIONAL MODELLING, UNIVERSITY OF WARSAW, WARSAW, POLAND

ABSTRACT

Aim: Type 2 diabetes mellitus (T2DM) is a widespread disease that leads to many complications if not adequately controlled. The pandemic and its limitations on healthcare access impaired the management of chronic conditions. The aim of our study was to examine its effects in context of different antidiabetic therapies on key health related factors in patients with T2DM.

Materials and Methods: To the study we enrolled 598 adult patients with diagnosed T2DM treated in diabetology outpatient department of the University Clinical Centre (UCC) of the Medical University of Warsaw. Data on body weight, glycated hemoglobin (HbA1c), and creatinine concentration were collected throughout the first COVID-19 pandemic wave and compared to the results obtained before the 4th of March, 2020 (1st confirmed COVID-19 case in Poland).

Results: The HbA1c mean baseline level was 7.15% (±1.39) and increased significantly (7.34% (±1.37), p=0.02) during observation. Importantly, the attendance of patients for HbA1c testing decreased by 57.82% in comparison to the pre-pandemic period. Similarly, creatinine concentrations increased (from 1.27 mg/dl (±0.76) to 1.34 mg/dl (±1.02), (p=0.004)). The increase in creatinine concentration was significantly lower in the group treated with regimens including metformin compared to other regimens. Somewhat surprisingly, the mean body mass remained unchanged.

Conclusions: The pandemic period had a significant impact on the tested biochemical parameters. The lesser changes of renal parameters in the group of patients treated with metformin confirms its nephroprotective effect and its value as a first-line treatment in T2DM.

KEY WORDS: diabetes mellitus, HbA1c, metformin, creatine, COVID-19 pandemic

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INTRODUCTION

Diabetes mellitus is a widespread disease that also has an impact on the onset of several severe complications, such as cardiovascular or renal issues [1]. Adequate treatment and monitoring of patients with type 2 diabetes mellitus (T2DM) are essential to reduce the risk of developing these complications.

Limited access to healthcare or modern forms of hypoglycemic therapy may have an impact on compliance with medical recommendations, directly affecting treatment outcomes. An example of the aforementioned scenario was encountered during the coronavirus disease 2019 pandemic, which led to a general decrease in the access to medical professionals, medication adherence and the management of chronic conditions [2, 3].

AIM

In our study, we aimed to examine the impact of limited access to healthcare during the COVID-19 pandemic on key health related factors in patients with T2DM - average body weight, creatinine levels, and glycated hemoglobin HbA1c levels in context of used antidiabetic therapies (including insulin, metformin, Sodium/glucose cotransporter-2 inhibitors (SGLT-2 inhibitors), and glucagon-like peptide-1receptor agonists (GLP-1 receptor agonists). Especially, we intended to investi-

Table 1. The baseline study population characteristics

Parameters	Number (percentage %)	Mean(±SD)
Age	598 (100)	68.8±11.87
Females	322 (53.85)	
Males	276 (46.15)	
Weight	559 (93.48)	87.09±19.54
Hypertension	481 (80.43)	
Atrial fibrillation	60 (10.03)	
Chronic coronary syndrome	131 (21.91)	
Dyslipidaemia	231 (38.63)	

Table 2. Treatment regimens

Medicine	Number (percentage %)
Metformine	428 (71.57)
Sulphonylourea	126 (21.07)
SGLT-2i	112 (18.73)
DPP-4i	47 (7.86)
GLP-1A	16 (2.68)
Other antidiabetic agent - acarbose, pioglitazone	11 (1.84)
Insulin	305 (51)

gate the specific impact of metformin on these variables in this setting, given its wider availability to patients compared to newer medications.

MATERIALS AND METHODS

STUDY DESIGN

The analysis involved 598 adult patients (322 females (53.85%) and 276 males (46,15%)) diagnosed with T2DM treated in the diabetology outpatient department at the University Clinical Centre (UCC) of the Medical University of Warsaw. Patients with type 1 diabetes mellitus were not included due to limited availability of clinical data (Table 1).

Clinical data were sourced from the UCC database and encompassed demographic characteristics (age and sex), weight, HbA1c levels, creatinine concentration, and antidiabetic agents. Blood samples were collected from patients using probes with an anticoagulant (EDTA), and all laboratory tests were conducted in the Central Laboratory of the UCC directly after sample collection. Creatinine concentration was measured using the Jaffé method (Roche), while HbA1c levels were assessed through capillary electrophoresis (Sebia). Measurements of weight, HbA1c, and creatinine were tracked throughout the initial pandemic wave until March 2021 and compared to data collected from September 2019 to 4th of March, 2020, the date which marked the first confirmed COVID-19 case in Poland). Antidiabetic treatment adhered to the guidelines of the Polish Diabetes Association [4], with patients receiving insulin, metformin (prescribed only in patients with eGFR>30 ml/min/1.73m2) SGLT-2 inhibitors, or other antidiabetic agents (Table 2).

STATISTICAL ANALYSIS

The data were analysed using Statistica 13 (TIBCO Software Inc., Palo Alto, CA, USA). To confirm the normal distribution of the data, the Lilliefors and Shapiro-Wilk tests were conducted. Analysis included Pearson and R-Spearman correlations, as well as one-way and two-way ANOVA and t-test comparisons. Statistical significance was established at a p-value less than 0.05.

STUDY ETHICS

This retrospective study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board at the Medical University of Warsaw was informed about the study (Approval ID: AKBE/162/2022) and the requirement for written informed consent from each patient was waived.

RESULTS

BODY WEIGHT

The mean body weight of patients before and during the pandemic did not show a significant change. There was no correlation between patient age and fluctuations of body weight. Moreover, various drug combinations were analysed for their impact on body weight. The sole instance where weight gain was noted was in patients concurrently receiving SGLT-2 inhibitors and insulin (p=0,03). No other notable variations in body weight were observed among patient groups categorized based on their prescribed therapies.

HBA1C CONCENTRATION

The HbA1c mean baseline level was 7.15% (±1.39) and increased significantly (7.34% (±1.37), p=0.02) during observation.

Upon segregating patients into distinct treatment groups, it was observed that none of the examined medications (metformin, insulin, SGLT-2 inhibitors) showed a more significant influence on this change compared to the others. There was no observable impact of patient age on the change in HbA1c concentration. Furthermore, none of the drug combinations showed a significant effect on the change in HbA1c levels.

Importantly, the attendance of patients for HbA1c testing decreased by 57.82% in comparison to the pre-pandemic period.

CREATININE LEVEL

The creatinine mean baseline concentration was 1.27mg/dl (±0.76) and increased significantly (1.34 mg/dl (±1.02); p=0.004) during observation. However, the increase in creatinine concentration was significantly lower in the group treated with regimens including metformin compared to other regimens (concentration change respectively 0.038 mg/dl vs 0.138 mg/dl, p=0.043). No other similar relationships were found, regardless of the treatment.

CORRELATIONS OF COMORBIDITIES

No significant correlations were found between the presence of comorbidities such as hypertension, atrial fibrillation, chronic coronary syndrome, dyslipidemia, and the monitored parameters (changes in creatinine level, body weight, and HbA1c levels), only body weight before the pandemic exhibited a positive correlation with the presence of dyslipidemia (Table 3-4).

DISCUSSION

Recent events, such as the COVID-19 pandemic and the current challenges in the pharmaceutical market have led to restricted access to medical services, affecting individuals with chronic conditions who require regular monitoring. Present situation in the pharmaceutical market is starting to resemble that of the pandemic due to the limited availability of certain medications, notably glucagon-like peptide-1 receptor agonists - globally overused for treatment of obesity. This scarcity may lead to metabolic changes akin to those observed in the population in 2020. Consequently, patients may be compelled to rely on accessible therapeutic agents.

For individuals with T2DM, maintaining consistent normoglycemia is essential in averting complications associated with diabetes. In our study, we focused on assessing basic metabolic parameters such as serum creatinine, HbA1c levels, and body weight due to their widespread availability enhancing the clinical applicability of the results, particularly in settings with limited resources.

Due to a reduction in access to healthcare services, in-person consultations decreased, leading to the adoption of telemedicine consultations during the pandemic. While basic assessments like self-monitored glucose profiles, signs of hyper/hypoglycemia, and body weight checks could be conducted by patients at home, these measures were often insufficient for a comprehensive evaluation of T2DM control and the implementation of essential therapeutic adjustments to mitigate the risk of diabetes-related complications. Certain critical tools required for these modifications, such as HbA1c and creatinine level measurements, necessitated in-person visits to healthcare facilities. Given the simplicity and widespread availability of these parameters, our study focused on monitoring changes in HbA1c, creatinine levels, and body weight.

In our study, somewhat surprisingly, throughout the observation period, the mean body weight remained stable within our group. This result is not consistent with the expectations and results of other researchers - a considerable number of studies have reported a notable weight gain during this timeframe [5]. It is possible that the lack of significance is due to the relatively small study group. Interestingly, we noted that the combined use of SGLT-2 inhibitors and insulin could potentially lead to weight gain, possibly due to the anabolic effects of insulin.

HbA1c serves as a widely accessible marker for assessing long-term glycemic control, reflecting the cumulative blood sugar levels over the previous two to three months. Its measurement is valuable in assessing the risk of diabetic complications, with even a single

Table 3. General results

Mean(±SD)	Starting point	Ending point	p
Body weight	87.09 kg	86.74 kg	0.105
HbA1c concentration	7.15 %	7.34 %	0.02
Creatinine level	1.27 mg/dl	1.34 mg/dl	0.004

Table 4. The change of measured parameters before and during the SARS-CoV-2 pandemic among patients who were not treated with metformin and treated with it

Delta (Δ)	Other agents	Metformin	p
Body weight	-0.393 kg	-0.207 kg	0.713
HbA1c concentration	0.213 %	0.004 %	0.277
Creatinine level	0.038 mg/dl	0.138 mg/dl	0.043

test offering reliable insights into diabetes diagnosis and prognosis [1].

In our study, a noteworthy increase in HbA1c levels was observed and deemed statistically significant, suggesting a dominant presence of factors associated with deteriorated overall glycemic control in our study population. Furthermore, the increase was similar in all treatment regimens. Additionally, no significant statistical correlation was found between the elevation in HbA1c levels and changes in body weight.

Our results are consistent with expectations, but scientific data from other authors on this subject are conflicting. Some studies, similarly to our data indicated that the lifestyle changes and reduced access to healthcare during the COVID-19 lockdown led to elevated HbA1c levels [6]. Conversely, other research has shown that in selected groups increased physical activity and high medication adherence during the pandemic contributed to lower HbA1c levels [7]. However, an American cohort study found no significant difference in HbA1c levels between 2019 and 2020 [8]. Our study supports the conclusion that the time of the pandemic negatively affected the control of type 2 diabetes in the study population.

Hyperglycemia contributes not only to cardiovascular complications but also to the development of structural and functional changes in the kidneys. Diabetic nephropathy is the leading cause of end-stage renal disease, so regular monitoring of creatinine levels for the assessment of kidney function in these patients is essential [9]. In our study, the creatinine mean baseline concentration increased significantly in the entire study group during observation. This finding, to the best of our knowledge, provides initial evidence that limited access to healthcare services during the COVID-19 pandemic contributed to a decline in kidney function among patients with T2DM. While the analysis of creatinine levels was conducted on 304 subjects (50.84%), the potential for bias due to limited data remains a concern. Nevertheless, the study offers insights into the progression of renal function during the SARS-CoV-2 pandemic. Interestingly, the decline in kidney function was less pronounced among patients receiving metformin compared to those not prescribed this medication. This outcome appears unexpected because other medications utilized within our clinical group also exhibit nephroprotective components.

Numerous clinical studies have highlighted the nephroprotective benefits of SGLT2 inhibitors and GLP-1 receptor agonists in individuals with normal or impaired glomerular filtration rate (GFR). Randomized trials involving patients with type 2 diabetes have shown that SGLT2 inhibitors can attenuate the decline in glomerular filtration rate, decrease the incidence of microalbuminuria, and slow or reverse the progression of proteinuria [10].

The CREDENCE study was a pioneering research focused on kidney disease, demonstrating benefits of SGLT2 inhibitors in patients with type 2 diabetes exhibiting substantial albuminuria and/or impaired kidney function. The primary composite endpoint related to kidney disease, encompassing end-stage kidney disease, doubling of serum creatinine, or renal death, was lowered by 34% [11].

Moreover, a new class of antidiabetic medications, such as GLP-1 receptor agonists, not only improve glycemic control and promote weight loss but also enhance cardiovascular and renal outcomes in patients with type 2 diabetes [12]. Studies in individuals with chronic kidney disease have demonstrated the safety of GLP-1 agonists in this population, showing no increased risk of acute kidney injury and effectiveness even at lower eGFR levels [13]. LEADER and SUSTAIN-6 studies identified a predefined renal composite, including crite-

ria such as new-onset or persistent macroalbuminuria, sustained doubling of serum creatinine, eGFR≤45 ml/min/1.73 m^2, the need for renal replacement therapy, and renal mortality. Liraglutide demonstrated a 22% reduction in this renal outcome, while semaglutide exhibited a 36% reduction [14, 15].

Despite the aforementioned results in numerous clinical trials, in our study the lack of discernible effects among other antidiabetic agents like SGLT-2 inhibitors or GLP-1 receptor agonists may be attributed to the limited number of patients using these medications, with financial constraints being a common reason for their reduced utilization. In Poland, reimbursement for SGLT-2 inhibitors or GLP-1 receptor agonist therapy is typically reserved for advanced cases of type 2 diabetes, making costly treatments financially burdensome for most patients.

Metformin remains a widely available option for patients, serving as the first-line oral therapy for individuals with T2DM [4]. This antihyperglycemic agent is extensively utilized in clinical practice. Historically, the use of metformin was discouraged in patients with acute or chronic kidney injury (AKI/CKD) due to concerns regarding the heightened risk of lactic acidosis [16]. Thus, metformin should not be prescribed for patients with advanced CKD, on account of an increased mortality risk related to metformin use in those patients [17]. On the other hand, a systemic review by Inzucchi et al. showed that when metformin was used in patients with mild to moderate CKD (eGFR 30–60 mL/min/1.73 m2), its serum levels usually remained within the therapeutic range [18].

Multiple studies presented the potential beneficial outcomes of metformin use in diabetic nephropathy and moderate CKD [19, 20]. The impact of metformin on the kidney in diabetics is also a consequence of its glucose-lowering-dependent mechanism [20]. Roussel et al. in a study that investigated the relationship between metformin use and mortality among T2DM patients with atherothrombosis found that metformin, prescribed in subjects with moderate renal failure (eGFR 30–60 mL/min/1.73 m^2), caused 36% risk reduction of mortality (HR 0.64; 95% CI, 0.48–0.86) [19]. Charytan et al. found that in patients in CKD, stage ≥4 metformin administration was associated with a decreased risk of kidney disease outcome, defined as an end-stage renal disease (ESRD) or death. The independent reductions of all-cause mortality were also reported [21]. Kwon et al. showed that metformin usage in advanced CKD patients, decreased the risk of all-cause mortality and incident ESRD by 35%[22].

Our results support the conclusions of other researchers about the nephroprotective effect of metformin and show that (perhaps due to high availability and low price) this effect is also visible in the population during a health care crisis. However, another possible explanation is that metformin was prescribed only in patients with eGFR>30 ml/min/1.73m^2. However, another possible explanation is that metformin was prescribed only in patients with normal to moderately decreased renal function. Another study showed that the decline of eGFR is greater in patients with decreased eGFR, thus the initial difference in eGFR level between the metformin group and non-metformin group may impact overall alternations in creatinine level [23].

There were several limitations to our study. Our research has a retrospective character. The number of patients included in our work was relatively small compared to similar studies. The time of observation was too short (less than two years) to analyse mortality and occurrence of cardiovascular events. Moreover, the time of the pandemic and the significant impact of SARS-CoV-2 infection on mortality and thromboembolic complications would make the interpretation of such results significantly more difficult.

Challenges in data collection arose from difficulties in maintaining contact with patients due to restricted personal interactions during the pandemic and obstacles encountered with lowered rate of participation in consultations.

We chose not to include patients with type 1 diabetes mellitus due to the limited availability of clinical data from this patient group for collection and analysis. Additionally, we reported results for each type of antihyperglycemic agent separately, even when patients were prescribed multiple medications from different groups. However, the number of patients receiving multidrug therapy involving the same categories of antihyperglycemic agents was insufficient to permit individual group analyses. Moreover, although other parameters like microalbuminuria might be more pertinent to diabetic nephropathy, we opted to use creatinine levels to assess renal function. This decision was influenced by the more routine nature of creatinine assessments as opposed to limited availability of microalbuminuria assessments in our study population prior to the COVID-19 pandemic.

Due to the retrospective nature our findings require further prospective studies.

CONCLUSIONS

The limited availability of healthcare services during the COVID-19 pandemic adversely affected the metabolic control of T2DM, evident through elevated levels of creatinine and HbA1c. This could potentially increase the long-term cardiovascular and renal risks for individuals. To mitigate these risks, there is a critical need for enhanced support

for patients and the formulation of preventive strategies that consider the most efficacious therapies in anticipation of future pandemic waves or pharmaceutical challenges.

Our study revealed that the rise in creatinine levels was notably lower in patients receiving metformin compared to those on other commonly prescribed antidiabetic medications. This underlines the nephroprotective potential of metformin, particularly during periods of suboptimal glycemic control or limited availability of modern antidiabetic treatments. It reinforces the importance of retaining metformin as a cornerstone in the treatment strategies for T2DM, emphasizing its value as a first-line therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

CORRESPONDING AUTHOR

Patrycja Małgorzata Bąk
Faculty of Medicine, Medical University of Warsaw,
02-091 Warsaw, Poland
e-mail: patrycjambak@gmail.com

ORCID AND CONTRIBUTIONSHIP

Marcin Kleibert: 0000-0003-1562-8450 A C D
Beata Mrozikiewicz-Rakowska: 0000-0002-1160-9204 A C D E F
Klaudia Korycka: 0009-0006-2542-6915 A C D E
Patrycja Małgorzata Bąk: 0000-0001-8259-0718 A B E F
Iga Płachta: 0000-0002-0830-2711 A C D
Daniel Balut: 0009-0003-2994-4400 A B
Wojciech Zgliczyński: 0000-0002-7283-4755 C D
Jakub Zieliński: 0000-0001-8935-8137 C D

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

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ORIGINAL ARTICLE

CONTENTS

Index assessment of periodontal status in patients on the background of administering a drug with circadian activity

Maryna Y. Vasko, Iryna M. Tkachenko, Yaroslav Y. Vodoriz, Anna V. Dvornyk, Oleh A. Pysarenko
POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Aim: The aim of this study was to determine the effect of application of drug with circadian activity (pioglitazone) for treatment of patients with periodontist.

Materials and Methods: Group I – 18 individuals with healthy periodontium. Group II – 12 participants with stage II, grade B periodontitis treated with a standard treatment protocol. Group III – 12 participants with stage II, grade B periodontitis, treated with a regimen that included the standard protocol along with the administration of pioglitazone prescribed at recommended times of intake. Group IV – 12 participants with stage II, grade B periodontitis treated with the standard protocol and pioglitazone against the recommended intake hours.

Results: The Simplified Oral Hygiene Index in Group I was 0.406±0.034. In Group II, it was 2.5±0.06. In Group III, the hygiene index was 2.633±0.056 and in Group IV it was 2.5±0.059.

The Papillary-Marginal-Alveolar Index in Group I was 0.033±0.004. In Group II, it was 0.366±0.011. For Group III, the PMA index was 0.38±0.012 and for Group IV it was 0.378±0.01.

The Russell's Periodontal Index in Group I was 0.111±0.008. In Group II, it was 4.668±0.155. For Group III – 4.708±0.132 and for Group IV it was 4.575±0.089.

The Gingival Bleeding Index (GBI) in Group I was 0.031±0.003, while in Group II, it was 0.266±0.009, in Group III, it was 0.273±0.007 and in Group IV it was 0.278±0.006.

Conclusions: The administration of pioglitazone (the drug with circadian activity) according to its circadian stage indeed has a positive effect on the periodontal status changes of patients with stage II, grade B periodontitis.

KEY WORDS: Periodontitis, Chronotype, Pioglitazone, Circadian Rhythm

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INTRODUCTION

Chronic generalized periodontitis arises due to the action of general and local factors and causes pathological changes that lead not only to the loss of basic masticatory functions but also induce general sensitization of the body to the action of microorganisms [1,2]. The damaged epithelium of the periodontal pockets, due to the inflammatory process, serves as entry gates through which antigens of bacterial origin penetrate into the general vascular system, causing bacteremia, which predisposes to the development of a whole range of somatic diseases [3,4].

In order to influence this link of the disease, it is expedient to prescribe drugs of general action that strengthen the vascular wall and restore its barrier function.

Pioglitazone is a drug that acts directly on several types of vascular cells: endothelial, smooth muscle cells of blood vessels, and macrophages. In endothelial cells, pioglitazone inhibits endothelial inflammation and monocyte adhesion and reduces endothelial dysfunction [5]. According to the principles of chronotherapy, all medicinal drugs have circadian dependence, namely the time of day when their effectiveness will be highest. The timing of medication intake depends directly on the interaction of hormones, among which glucocorticoids, catecholamines, glucagon, somatotropic hormone, thyroid hormones play the most significant role, each of which has its own daily secretion rhythms with acrophases covering almost the entire day. Pioglitazone also is a highly selective γ-PPAR agonist that modulates transcription of some insulin-sensitive genes involved in glucose control and lipid metabolism. It is known that the drug able to influence the activity of peripheral molecular components watches, also it is a highly selective γ-PPAR agonist that modulates transcription of some insulin-sensitive genes involved in glucose control and lipid metabolism. It is known that the drug able to influence the activity of peripheral molecular components watches

Since the main action of pioglitazone is antidiabetic, morning administration of this drug is associated with the so-called "dawn phenomenon" [6], which manifests itself in

patients with diabetes and consists of an increase in blood glucose levels in the early morning hours. Thus, prescribing the drug according to its circadian stage will allow achieving maximum effectiveness, and its administration at other times of the day will completely neutralize any effect [7,8].

AIM

The aim of this study was to determine the effect of application of drug with circadian activity (pioglitazone) for treatment of patients with periodontist.

MATERIALS AND METHODS

For the study, an age group of 36-45 years was selected, namely middle-aged individuals according to the WHO age classification, totaling 54 subjects.

Group I (control group) included 18 individuals with healthy periodontium and the absence of any somatic pathology or compensated somatic pathology. Group II comprised 12 participants diagnosed with stage II, grade B periodontitis, treated with a standard treatment protocol. Group III consisted of 12 participants diagnosed with stage II, grade B periodontitis, treated with a regimen that included the standard protocol along with the administration of pioglitazone at a dose of 30mg for 28 days, prescribed at recommended times of intake, specifically in the morning hours. Group IV was composed of 12 participants diagnosed with stage II, grade B periodontitis, treated with the standard protocol and pioglitazone at a dose of 30mg for 28 days against the recommended intake hours, specifically in the evening hours.

Patient selection was conducted using inclusion and exclusion criteria.

Inclusion criteria for clinical study:

1. Signed informed consent.
2. Presence of a minimum of 19 teeth.
3. Presence of periodontitis (periodontal pockets up to 4.5-5mm).
4. Individuals without diagnosed somatic pathology or with compensated somatic pathology.
5. Age of patients: 36-45 years.

Exclusion criteria from the study:

1. Use of antibiotics or anti-inflammatory drugs within the previous 3 months.
2. Periodontal therapy within the previous 6 months.
3. Purulent exudation from periodontal pockets.
4. Pregnancy or breastfeeding.
5. Presence of severe, uncontrolled (decompensated) diseases of internal organs or neuro-psychiatric disorders.
6. Presence of other conditions determining the patient's inability to understand the nature and possible consequences of the study.
7. Inability for prolonged observation.

After comprehensive examination and diagnosis establishment of the disease stage, all patients were prescribed standard local periodontal treatment divided into several consecutive phases. A treatment plan was prepared beforehand, discussed, and agreed upon with the patient.

PHASE 1

During the initial stage, oral cavity sanitation was performed, including the removal of local traumatic factors such as carious cavities, overhanging margins of fillings, inadequately fabricated orthopedic constructions and prostheses, traumatic occlusion, malocclusion, and dental deposits. Professional oral hygiene procedures were carried out, involving the removal of supragingival and subgingival dental deposits using an ultrasonic scaler and manual instruments, and teeth polishing using Air-Flow abrasive treatment with Air-Flow Perio powder by EMS. To alleviate clinical symptoms of the disease, irrigation with 0.05% chlorhexidine solution and application of "Metrogyl-Denta" gel on the gums were performed. Patients were educated on oral hygiene practices and provided with personalized hygiene aids and tools. Local medication therapy included rinsing with 0.05% chlorhexidine solution twice a day for 7 days and application of "Metrogyl-Denta" gel on the gums twice a day for 7 days.

After 3 months, tissue healing and periodontal tissue condition were assessed, as this period is sufficient for tissue regeneration after therapeutic interventions [9]. If necessary, repeated subgingival instrumentation was performed.

PHASE 2

After 6 months, the response of periodontal tissues to periodontal therapy was evaluated, as this timeframe allowed for adequate assessment of changes in bone tissue and decision-making regarding further patient management [9]. Subsequently, patients were subjected to regular follow-up appointments - four times a year.

PHASE 3

Restorative treatment and permanent prosthetic restoration with the use of splints and splint-prostheses were carried out as indicated.

PHASE 4

Following active periodontal therapy, comprehensive supportive therapy was implemented, including oral hygiene instructions, repeated removal of dental deposits, root surface treatment, and surgical interventions as indicated.

The effectiveness of the treatment was evaluated by determining periodontal indices PMA, PI, BOP, and oral hygiene index OHI-S [10].

The quantitative indicators obtained during the patient examinations were input into an Excel spreadsheet and processed using methods of mathematical statistics to calculate the sample means (M), standard deviation (σ), and standard errors of the means (m) within the examined groups. Frequency tables were constructed for semi-quantitative and qualitative indicators [11].

Table 1. Average values of index indicators of patients of the studied groups before the start of treatment

Index	Group			
	I	II	III	IV
OHI-S	0,406±0,034	2,5±0,06	2,633±0,056	2,5±0,059
PMA	0,033±0,004	0,366±0,011	0,38±0,012	0,378±0,01
PI	0,111±0,008	4,668±0,155	4,708±0,132	4,575±0,089
BOP	0,031±0,003	0,266±0,009	0,273±0,007	0,278±0,006

The statistical significance of differences between the obtained results for different groups was determined using the non-parametric Mann-Whitney U test. The likelihood of differences between indicators at different times of the day was determined using the Wilcoxon method for paired samples [10]. Differences were considered significant at $p < 0.01$. Computations were performed using the "Microsoft Excel 2007" and "SPSS for Windows, Release 13.0" software.

The likelihood of differences in the obtained results between different groups was determined using analysis of variance (ANOVA). Differences were considered significant at the generally accepted probability of error $p < 0.05$ in medical-biological research.

RESULTS

The Simplified Oral Hygiene Index (OHI-S) among patients in Group I was 0.406±0.034, corresponding to a good level of oral hygiene. Among patients in Group II, it was 2.5±0.06, indicating an unsatisfactory level of hygiene. In Group III, the hygiene index was 2.633±0.056, and in Group IV, it was 2.5±0.059, both corresponding to an unsatisfactory level of oral hygiene.

The Papillary-Marginal-Alveolar Index (PMA) among patients in Group I was 0.033±0.004, indicating a mild degree of gingivitis. In Group II, it was 0.366±0.011, representing a moderate degree of gingivitis. For Group III, the PMA index was 0.38±0.012, and for Group IV, it was 0.378±0.01, both indicating a moderate degree of gingivitis.

The Russell's Periodontal Index (PI) among patients in Group I was 0.111±0.008, indicating an initial stage of periodontal pathology. In Group II, it was 4.668±0.155, indicating a severe stage of periodontal pathology. For Group III, the PI was 4.708±0.132, and for Group IV, it was 4.575±0.089, both indicating a severe stage of periodontitis.

The Gingival Bleeding Index (GBI) among patients in Group I was 0.031±0.003, while in Group II, it was 0.266±0.009, in Group III, it was 0.273±0.007, and in Group IV, it was 0.278±0.006 (Table 1).

These results indicate significant differences in oral hygiene, gingival health, and severity of periodontal disease among the examined groups. Statistical analysis using the Mann-Whitney U test and ANOVA confirmed the significance of these differences ($p < 0.01$). The findings suggest the effectiveness of the treatment protocols employed in improving oral health parameters, particularly in Groups I and III, where better outcomes were observed.

The Oral Hygiene Index (OHI-S) among subjects in Group I changed from 0.406±0.034 to 0.411±0.036 over the course of 1 year. The value of the Papillary-Marginal-Alveolar Index (PMA) changed from 0.033±0.004 to 0.035±0.005 within the same period. The Russell's Periodontal Index (PI) remained unchanged at 0.111±0.008. The Gingival Bleeding Index (GBI) changed from 0.031±0.003 to 0.031±0.004 after 1 year. Since the subjects in Group I had healthy periodontal tissues, the index values remained practically unchanged after 1 year (Table 2).

The Oral Hygiene Index (OHI-S) among patients in Group II changed from 2.5±0.06 before treatment to 0.508±0.042 after 3 months, indicating a good level of hygiene, to 0.492±0.026 after 6 months, still indicating a good level of hygiene, and to 0.425±0.022 after 1 year, also indicating a good level of hygiene. The changes at 3 months, 6 months, and 1 year were statistically significant ($p < 0.001$).

The Papillary-Marginal-Alveolar Index (PMA) among patients in Group II decreased from 0.366±0.011 before treatment to 0.079±0.002 after 3 months, indicating a mild level of gingivitis, to 0.091±0.006 after 6 months, still indicating a mild level of gingivitis, and to 0.081±0.012 after 1 year, also indicating a mild level of gingivitis. The results were statistically significant ($p < 0.001$).

The Russell's Periodontal Index among patients in Group II changed from 4.668±0.155 before treatment to 0.850±0.036 after 3 months, indicating a mild severity of periodontitis, to 0.917±0.063 after 6 months, still indicating a mild severity of periodontitis, and to 0.775±0.093 after 1 year, also indicating a mild severity of periodontitis. The changes at 3 months, 6 months, and 1 year were statistically significant ($p < 0.001$).

The Gingival Bleeding Index among patients in Group II decreased from 0.266±0.009 before treatment to 0.066±0.001 after 3 months, to 0.073±0.003 after 6 months, and to 0.067±0.005 after 1 year, which was statistically significant ($p < 0.001$) (Table 3).

Table 2. Average values of index indicators of the I group after 1 year of observation

Terms / Indices	Before treatment	1-year follow-up	p-value
OHI-S	0,406±0,034	0,411±0,036	0,889
PMA	0,033±0,004	0,035±0,005	0,708
PI	0,111±0,008	0,111±0,008	1
BOP	0,031±0,003	0,031±0,004	0,819

Table 3. Average values of index indicators of the II group after 1 year of observation

Terms / Indices	Before treatment	3 months follow-up	p_1	6 months follow-up	p_2	1-year follow-up	p_3
OHI-S	2,5± 0,06	0,508± 0,042	< 0,001	0,492± 0,026	< 0,001	0,425± 0,022	< 0,001
PMA	0,366± 0,011	0,079± 0,002	< 0,001	0,091± 0,006	< 0,001	0,081± 0,012	< 0,001
PI	4,668± 0,155	0,850± 0,036	< 0,001	0,917± 0,063	< 0,001	0,775± 0,093	< 0,001
BOP	0,266± 0,009	0,066± 0,001	< 0,001	0,073± 0,003	< 0,001	0,067± 0,005	< 0,001

Table 4. Average values of index indicators of patients of the III group after 1 year of observation

Terms / Indices	Before treatment	3 months follow-up	p_1	6 months follow-up	p_2	1-year follow-up	p_3
OHI-S	2,633± 0,056	0,500± 0,033	< 0,001	0,433± 0,019	< 0,001	0,392± 0,026	< 0,001
PMA	0,38± 0,012	0,081± 0,002	< 0,001	0,076± 0,005	< 0,001	0,065± 0,002	< 0,001
PI	4,708± 0,132	0,832± 0,019	< 0,001	0,725± 0,045	< 0,001	0,625± 0,041	< 0,001
BOP	0,273± 0,007	0,067± 0,002	< 0,001	0,063± 0,002	< 0,001	0,058± 0,002	< 0,001

The Oral Hygiene Index (OHI-S) among patients in Group III changed from 2.633±0.056 before treatment to 0.500±0.033 after 3 months, indicating a good level of hygiene, to 0.433±0.019 after 6 months, still indicating a good level of hygiene, and to 0.392±0.026 after 1 year, also indicating a good level of hygiene. The changes in the indices at 3 months, 6 months, and 1 year were statistically significant ($p < 0.001$).

The Papillary-Marginal-Alveolar Index (PMA) among patients in Group III decreased from 0.38±0.012 before treatment to 0.081±0.002 after 3 months, indicating a mild level of gingivitis, to 0.076±0.005 after 6 months, still indicating a mild level of gingivitis, and to 0.065±0.002 after 1 year, also indicating a mild level of gingivitis. The results were statistically significant ($p < 0.001$).

The Russell's Periodontal Index among patients in Group III changed from 4.708±0.132 before treatment to 0.832±0.019 after 3 months, indicating a mild severity of periodontitis, to 0.725±0.045 after 6 months, still indicating a mild severity of periodontitis, and to 0.625±0.041 after 1 year, also indicating a mild severity of periodontitis. The changes in the indices at 3 months, 6 months, and 1 year were statistically significant ($p < 0.001$).

The Gingival Bleeding Index among patients in Group III decreased from 0.273±0.007 before treatment to 0.067±0.002 after 3 months, to 0.063±0.002 after 6 months, and to 0.058±0.002 after 1 year, which was statistically significant ($p < 0.001$) (Table 4).

The Oral Hygiene Index (OHI-S) among patients in Group IV changed from 2.5±0.059 before treatment to 0.542±0.034 after 3 months, indicating a good level of hygiene, to 0.492±0.026 after 6 months, still indicating a good level of hygiene, and to 0.383±0.024 after 1 year, also indicating a good level of hygiene. The changes in the indices at 3 months, 6 months, and 1 year were statistically significant ($p < 0.001$).

Table 5. Average values of index indicators of IV group patients after 1 year of observation

Terms / Indices	Before treatment	3 months follow-up	p_1	6 months follow-up	p_2	1-year follow-up	p_3
OHI-S	2,5± 0,059	0,542± 0,034	< 0,001	0,492± 0,026	< 0,001	0,383± 0,024	< 0,001
PMA	0,378± 0,01	0,082± 0,002	< 0,001	0,081± 0,004	< 0,001	0,077± 0,009	< 0,001
PI	4,575± 0,089	0,825± 0,041	< 0,001	0,833± 0,051	< 0,001	0,675± 0,071	< 0,001
BOP	0,278± 0,006	0,067± 0,001	< 0,001	0,067± 0,003	< 0,001	0,064± 0,003	

The Papillary-Marginal-Alveolar Index (PMA) among patients in Group IV decreased from 0.378±0.01 before treatment to 0.082±0.002 after 3 months, indicating a mild level of gingivitis, to 0.081±0.004 after 6 months, still indicating a mild level of gingivitis, and to 0.077±0.009 after 1 year, also indicating a mild level of gingivitis. The results were statistically significant ($p < 0.001$).

The Russell's Periodontal Index among patients in Group IV changed from 4.575±0.089 before treatment to 0.825±0.041 after 3 months, indicating a mild severity of periodontitis, to 0.833±0.051 after 6 months, still indicating a mild severity of periodontitis, and to 0.675±0.071 after 1 year, also indicating a mild severity of periodontitis. The changes in the indices at 3 months, 6 months, and 1 year were statistically significant ($p < 0.001$).

The Gingival Bleeding Index among patients in Group IV decreased from 0.278±0.006 before treatment to 0.067±0.001 after 3 months, to 0.067±0.003 after 6 months, and to 0.064±0.003 after 1 year, which was statistically significant ($p < 0.001$) (Table 5).

DISCUSSION

Since the subjects in Group I had healthy periodontal tissues, the index values remained practically unchanged after 1 year

The improvement of periodontal status of patients of the II group could be explained with the proper antimicrobial and biofilm management both chairside and homecare. Proper professional hygiene and administration of gel which contain drugs that are considered to be the "golden standard" against perio-pathogenes (metronidazole and chlorhexidine) lead to good results and improvement of periodontal health.

The administration of pioglitazone in the III research group has demonstrated a significant positive effect of periodontal status of patients. The effectiveness of the drug can be explained of its circadian activity. The morning prescription is which is related the "dawn phenomenon" [6], which consists of an increase in blood glucose levels in the early morning hours. Thus, prescribing the drug that modulates transcription of some insulin-sensitive genes involved in glucose control and lipid metabolism according to its circadian stage will lead to more effective treatment [7,8].

The obtained results indicate significant differences in oral hygiene, gingival health, and severity of periodontal disease among patients of the examined groups. Statistical analysis using the Mann-Whitney U test and ANOVA confirmed the significance of these differences ($p < 0.01$). The findings suggest the effectiveness of the treatment protocols employed in improving oral health parameters, particularly in Groups I and III, where better outcomes were observed.

Thus, analyzing the obtained results, we notice that Group II, which received the standard treatment protocol, demonstrated the worst dynamics compared to Groups III and IV in terms of the physical health (PH) quality of life scale, PMA index, PI index, GI index, depth of periodontal pockets, and loss of epithelial attachment. Group IV, which, in addition to standard treatment, received pioglitazone against recommended timing, showed the best dynamics in terms of the physical health (PH) quality of life scale, depth of periodontal pockets, and loss of epithelial attachment. And Group III, which, in addition to standard treatment, received pioglitazone at recommended timing, showed the best dynamics in terms of the PMA index, PI index, GI index. These indices are directly related to or characterize the severity of gingival bleeding. Therefore, it can be assumed that the administration of pioglitazone according to its circadian stage indeed has a positive effect on the vascular system, specifically on vascular permeability and endothelial function.

CONCLUSIONS

Therefore, it can be concluded that the administration of pioglitazone (the drug with circadian activity) according to its circadian stage indeed has a positive effect on the periodontal status changes of patients with stage II, grade B periodontitis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Iryna M. Tkachenko
Poltava State Medical University
23 Shevchenko st, 36000 Poltava, Ukrain
e-mail: tkachenkoirmix@gmail.com

ORCID AND CONTRIBUTIONSHIP

Maryna Y. Vasko: 0000-0003-2164-7076 A B E
Yaroslav Y. Vodoriz:. 0000-0001-9388-1270 C D
Anna V. Dvornyk: 0000-0001-6297-0609 B D
Oleh A. Pysarenko: 0000-0002-6104-6745 A E
Iryna M. Tkachenko: 0000-0001-8243-8644 A E F

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

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ORIGINAL ARTICLE

CONTENTS

Morphological and functional features of the colonic mucus barrier in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis

Hanna A. Dorohavtseva[1], Andrey E. Dorofieiev[2], Olena O. Dyadyk[2], Mykhailo S. Myroshnychenko[3]

[1] FEOFANIYA CLINICAL HOSPITAL OF THE STATE ADMINISTRATION OF AFFAIRS, KYIV, UKRAINE

[2] SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

[3] KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Aim: The purpose was to identify the morphological and functional features of the colonic mucus barrier in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

Materials and Methods: In the research, three groups were formed. Group 1 included fragments of the mucous membrane of the large intestine, which were collected from 12 people during autopsies. The results of autopsies and histological examination of the material did not reveal any gastrointestinal pathology. Group 2 included biopsies of the mucous membrane of the large intestine from the area of the diverticulum of 34 patients with symptomatic uncomplicated diverticular disease. Group 3 included biopsies of the mucous membrane of the large intestine of 26 patients with acute uncomplicated diverticulitis. Histological (hematoxylin and eosin staining), histochemical (PAS reaction) and immunohistochemical (mouse monoclonal antibodies to Mucin 2 (MUC2) and Mucin 4 (MUC4)) staining methods were used. A morphometric study was also carried out.

Results: In patients with diverticular disease, the authors identified disturbances in the morphofunctional state of the mucus barrier of the colon, the structure and function of goblet cells contained in its mucous membrane, characterized by a decrease in the thickness of the mucus layer covering the surface of the mucous membrane; a decrease in the size and number of goblet cells with a decrease in their mucus-producing ability; a change in the mucin profile, characterized by a violation of the content of MUC2 and MUC4. These changes were greatest in patients with acute uncomplicated diverticulitis compared with patients with symptomatic uncomplicated diverticular disease.

Conclusions: The identified disturbances in the morphofunctional state of the mucus barrier of the colon, structural and functional changes in goblet cells may be one of the mechanisms for the development of acute uncomplicated diverticulitis and symptomatic uncomplicated diverticular disease.

KEY WORDS: colonic mucus barrier, symptomatic uncomplicated diverticular disease, acute uncomplicated diverticulitis, morphological and functional features

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INTRODUCTION

The large intestinal epithelia are covered by a mucus layer which consists of about 30 core proteins, including mucins, antimicrobial peptides, secreted immunoglobulin A [1, 2]. It is a viscous gel-like layer, forming inner firm and outer loose mucus layers, moreover, the latter accounts for more than 80% of the thickness [3-5]. This layer has a primary role in intestinal protection against mechanical, chemical, biological attacks and contributes to the maintenance of intestinal homeostasis [6].

Among the intestinal mucus layer components, mucins are the most important [1]. Goblet cells are single-cell glands that produce and secrete mucins. It is known that mucin is a high-molecular-weight glycoprotein, the synthesis process of which includes dimerization in the endoplasmic reticulum, Golgi glycosylation, and finally oligomerization [7]. There are currently 22 known mucins which named from MUC1 through MUC22 [8]. All mucins are divided into membrane-associated (MUC1, MUC3A/B, MUC4, MUC12, MUC13, MUC15, MUC17, MUC20, MUC21), which perform a protective function and take part in intracellular signal transmission, and secretory. Secretory mucins are categorized into two subclasses: gel-forming and nongelforming mucins. MUC2, MUC5AC, MUC5B, MUC6 and MUC19 are gel-forming mucins involved in protection, transportation, lubrication, and hydration, and MUC7, MUC8, MUC9 are nongel-forming mucins [8, 9]. MUC1, MUC2, MUC3 and MUC4 are the predominant mucins detected in the large intestine mucous membrane [10].

The lesion, abnormal proliferation and differentiation of goblet cells, as well as the deficiency synthesis and

secretion of mucins, result in intestinal mucosal barrier dysfunction [11]. The latter is an important determinant of intestinal diseases (inflammatory bowel disease, irritable bowel syndrome, colorectal cancer), disorder in extraintestinal organs [8].

Diverticular disease is a common, gradually progressive chronic gastrointestinal condition with increasing prevalence which manifests in early adulthood and progresses with advancing age with respect to its anatomical extent and diverticula size [12, 13]. Disturbances in the morphological and functional state of the colon mucus layer may be one of the mechanisms for the development of various variants of diverticular disease. There is no information in the literature about the morphological features of the colon mucus barrier in patients with diverticular disease, which makes this study relevant.

AIM

The purpose was to identify the morphological and functional features of the colonic mucus barrier in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

MATERIALS AND METHODS

In the research, three groups were formed. Group 1 included fragments of the mucous membrane of the large intestine, which were collected from 12 people during autopsies on the basis of the Pathology Department of the Municipal Non-Profit Enterprise of the Kyiv Regional Council «Kyiv Regional Clinical Hospital». The results of autopsies and histological examination of the material did not reveal any gastrointestinal pathology. Group 2 included biopsies of the mucous membrane of the large intestine from the area of the diverticulum of 34 patients with symptomatic uncomplicated diverticular disease. Group 3 included biopsies of the mucous membrane of the large intestine of 26 patients with acute uncomplicated diverticulitis. Patients of groups 2 and 3 were treated in the Gastroenterology Department of the Feofaniya Clinical Hospital of the State Administration of Affairs for the period from 2019 to 2022.

Biopsies of the colon mucosa were fixed in a 10% solution of neutral buffered formalin (pH 7.4) for 24-48 hours. After fixation, the material was processed according to the standard method in an Excelsior AS apparatus (Thermo Fisher Scientific, UK), embedded in paraffin blocks on a HistoStar apparatus (Thermo Fisher Scientific, UK), from which serial histological sections with a thickness of 2-3 μm were prepared. The latter were stained with hematoxylin and eosin. PAS reaction was performed.

An immunohistochemical study was performed on Super Frost Plus adhesive slides (Menzel, Germany). For high-temperature processing of epitopes of antigens, citrate buffer with pH 6 and EDTA buffer (pH 8) were used. The UltraVision Quanto HRP detection system, DAB Quanto chromogen manufactured by Thermo Fisher Scientific (USA) was used. Immunohistochemical study was performed using mouse monoclonal antibodies to MUC2 (clone Ccp58, Master Diagnostica, Spain), MUC4 (clone 8G7, Master Diagnostica, Spain).

Hematoxylin and eosin-stained microscope specimens were examined using a ZEISS Primostar 3 microscope (Carl Zeiss, Germany) with a built-in digital color camera, a BRESSER Science TFM-301 Trino microscope with a BRESSER Full HD camera (Bresser GmbH, Germany).

Using the Labscope program, a morphometric study was carried out, during which in groups 1-3 it was determined the thickness of the layer of PAS-positive secret located above the surface epithelium of the colon mucosa in the field of view of the microscope ×400; the absolute number of goblet cells in the intestinal glands in the field of view of the microscope ×200. The severity of the PAS reaction in goblet cells of the intestinal glands, the expression of MUC2 and MUC4 in the epithelium of the intestinal glands and surface epithelium were assessed in the field of view of the microscope ×400 by determining the brightness coefficient in the Lab color model using the computer program "Analysis of color properties of raster images" [14, 15].

Indicators in groups 1-3 were processed statistically using the PAST program (version 4.15, Natural History Museum, University of Oslo, Norway). Average values of indicators in groups were compared using the Student's t-test and the Mann-Whitney U-test. Differences of indicators were considered significant at $p<0.05$.

RESULTS

When performing a PAS reaction it was noted in all groups in the mucous membrane of the colon a PAS-positive secret located in the form of a layer above the surface epithelium; in goblet cells of the surface epithelium and intestinal glands of varying degrees of severity.

In group 1, a uniformly expressed layer of PAS-positive secretion was determined over the surface epithelium (Fig. 1), the average thickness of which was (12.08±0.59) μm (Fig. 2). In group 2 and, especially, in group 3, an unevenly expressed layer of PAS-positive secretion was detected above the surface epithelium of the mucous membrane. In group 3, in the part of the visual fields where erosive and ulcerative changes were determined, this layer was not identified. The average thickness of

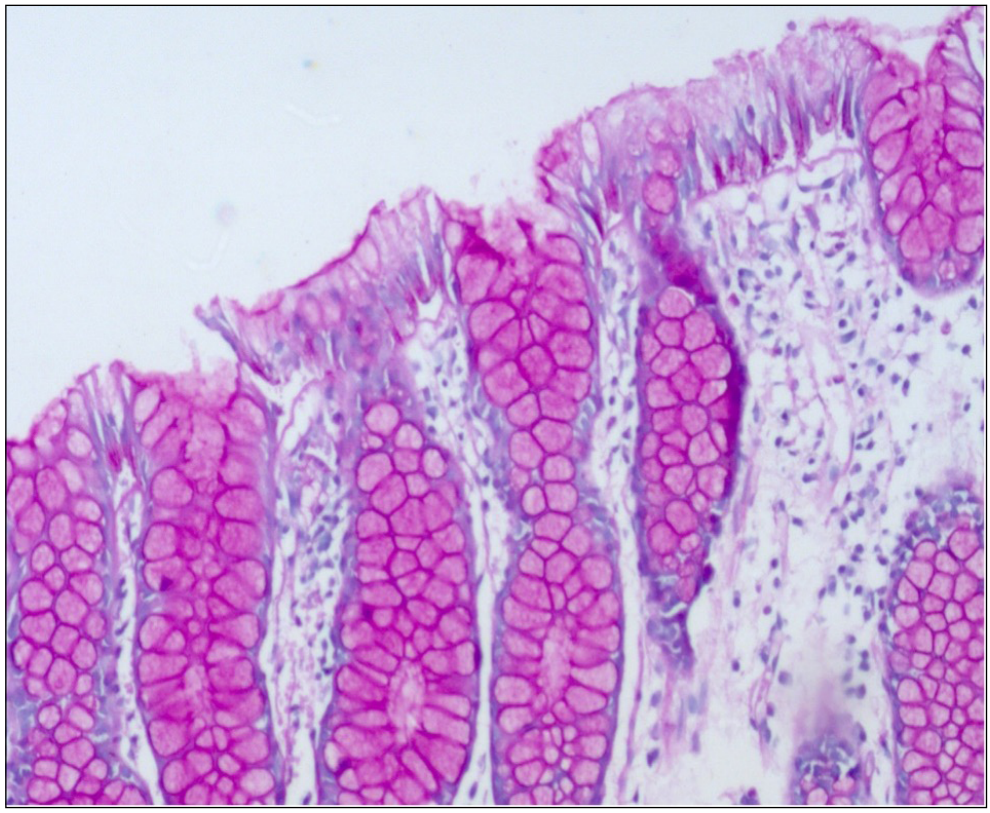

Fig. 1. Group 1. A uniformly expressed layer of PAS-positive secretion over the surface epithelium. PAS-positive reaction in goblet cells of the colon mucosa in the form of their uniform staining. PAS reaction, × 100.

this layer in group 3 was significantly ($p<0.05$) smaller than in group 2 (Fig. 2). The thickness of the PAS-positive secretion layer in groups 2 and 3 was significantly ($p<0.05$) smaller than the corresponding indicator in group 1.

During the subsequent analysis of the PAS reaction, the morphofunctional state of goblet cells in groups 1-3 was assessed. In all groups, it was identified a pattern of distribution of goblet cells in the surface epithelium and epithelium of the intestinal glands. Thus, in the epithelium of the intestinal glands the content of goblet cells was high.

In group 1, in the surface epithelium and intestinal glands, goblet cells were characterized by the presence of one or two vacuoles filled with evenly distributed PAS-positive secretion, the average brightness coefficient of which was 0.77±0.005 (Fig. 3). This secretion was also detected in the lumen of the intestinal glands. The average absolute number of goblet cells in the intestinal glands was 43.83±1.03 (Fig. 4).

In group 2, the number of goblet cells in the intestinal glands was significantly ($p<0.05$) lower compared to group 1 (Fig. 4). Also, compared with group 1, goblet cells in group 2 were characterized by smaller sizes and the content of a larger number of small vacuoles. The latter contained PAS-positive secretion or granules of varying intensity of staining. The average value of the brightness coefficient in the vacuoles of goblet cells was significantly ($p<0.05$) higher compared to group 1, which indicated a decrease in the ability of these cells to mucus produce (Fig. 3).

In group 3, compared to group 1, goblet cells were smaller and contained several small vacuoles (Fig. 5). The latter contained PAS-positive secretion or granules. When performing the PAS reaction, only the contours of the vacuoles were stained in most fields of view. In such goblet cells, survey microscopy revealed atrophic and degenerative changes of varying severity. The average value of the brightness coefficient in the vacuoles of goblet cells was significantly ($p<0.05$) greater than the corresponding indicators of groups 1 and 2, which indicated inhibition of mucus secretion by these cells (Fig. 3). In group 3, the average value of the absolute number of goblet cells in the intestinal glands was significantly ($p<0.05$) lower compared to the corresponding indicators of groups 1 and 2 (Fig. 4).

In groups 1-3, an immunohistochemical reaction with monoclonal antibodies to MUC2 and MUC4 revealed their membrane expression in the surface epithelium and epithelium of the intestinal glands. In group 1, the expression of MUC2 and MUC4 was uniformly expressed (Fig. 6). In group 2 and, especially, in group 3, uneven and reduced expression of these monoclonal antibodies was determined (Fig. 7).

The results of determining the brightness coefficient in immunohistochemical reactions with monoclonal antibodies to MUC2 and MUC4 are shown in Table 1. The table shows that the indicators of groups 2 and

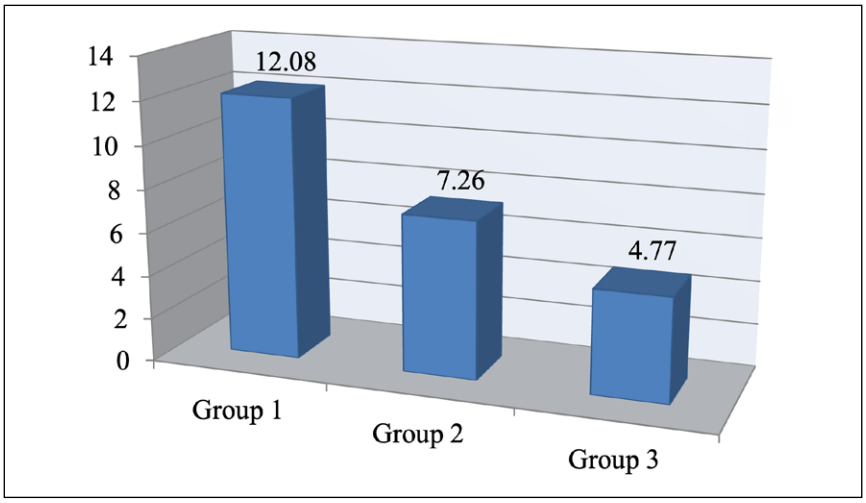

Fig. 2. Average values of the thickness of the PAS-positive secretion layer on the surface of the colon mucosa in groups 1-3.

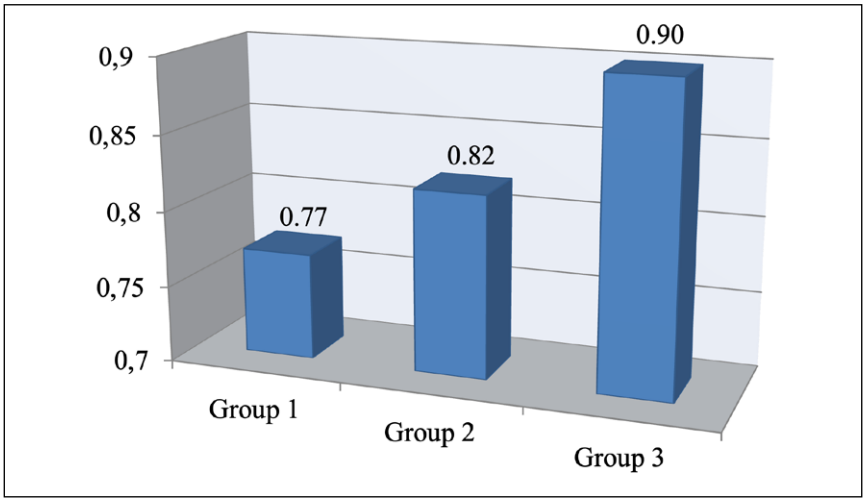

Fig. 3. Average values of the brightness coefficient of PAS-positive secretion in goblet cells in groups 1-3.

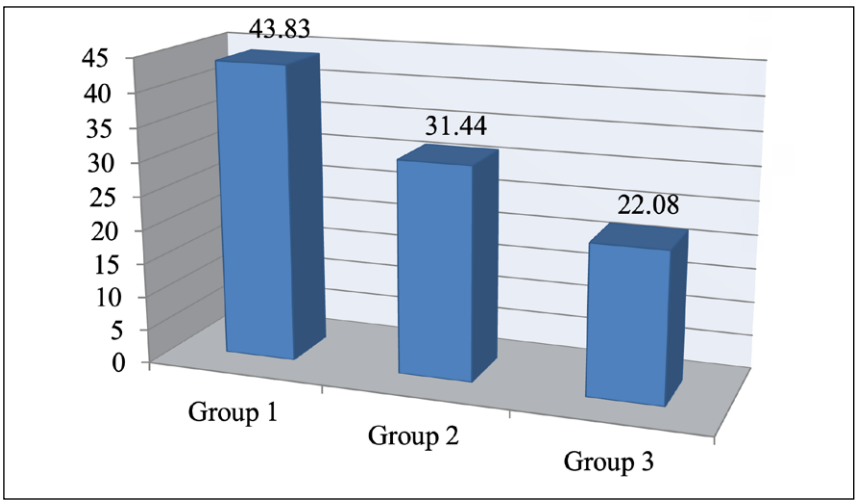

Fig. 4. Average values of the absolute number of goblet cells in the intestinal glands in groups 1-3.

3 were significantly ($p<0.05$) increased compared to group 1, which indicated a decrease in the expression of these monoclonal antibodies. Moreover, the most pronounced decrease in the expression of MUC2 and MUC4 was observed in group 3 compared to group 2.

It is interesting that in groups 1 and 2 the brightness coefficient took a significantly ($p<0.05$) lower value in the reaction with MUC4 compared to the reaction with MUC2, which indicated a higher content of MUC4. In group 3, no such dependence was detected, which indicated an equal content of such mucins in the mucus.

DISCUSSION

In a comprehensive morphological study conducted by the authors, for the first time, disturbances in the morphofunctional state of the mucus barrier and goblet cells in the colon in patients with symptomatic uncom-

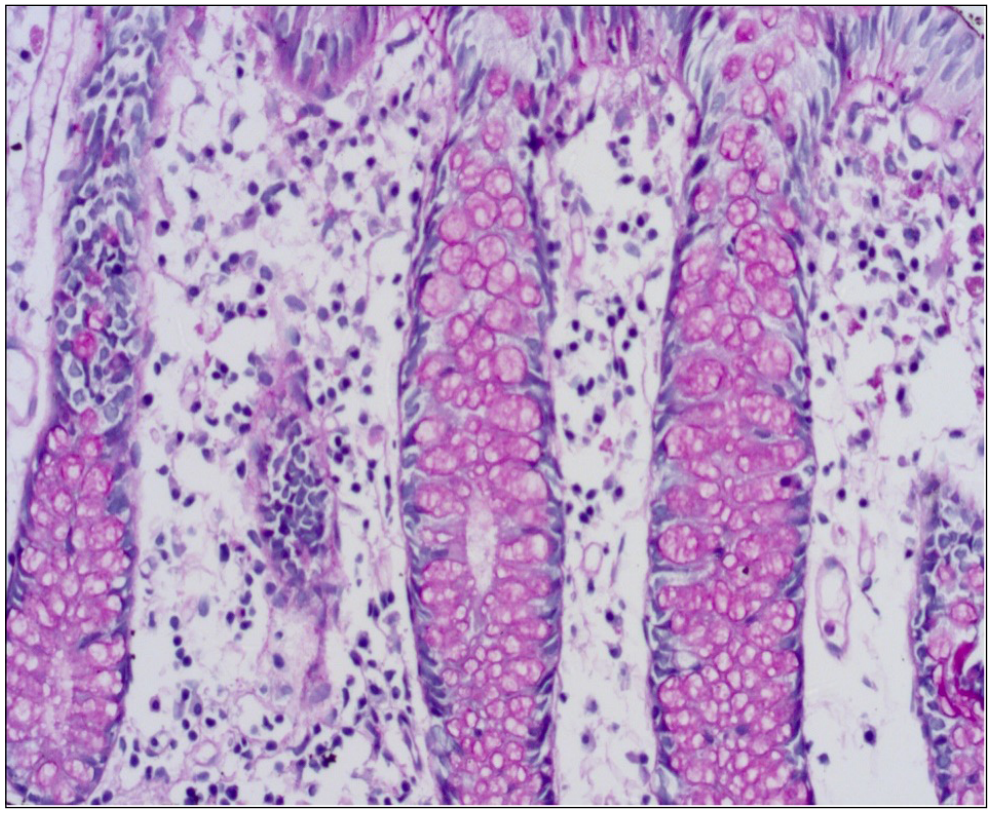

Fig. 5. Group 3. Secretions or granules in goblet cells of varying intensity of staining. Staining in part of the goblet cells only the outline of the vacuoles. PAS reaction, × 200.

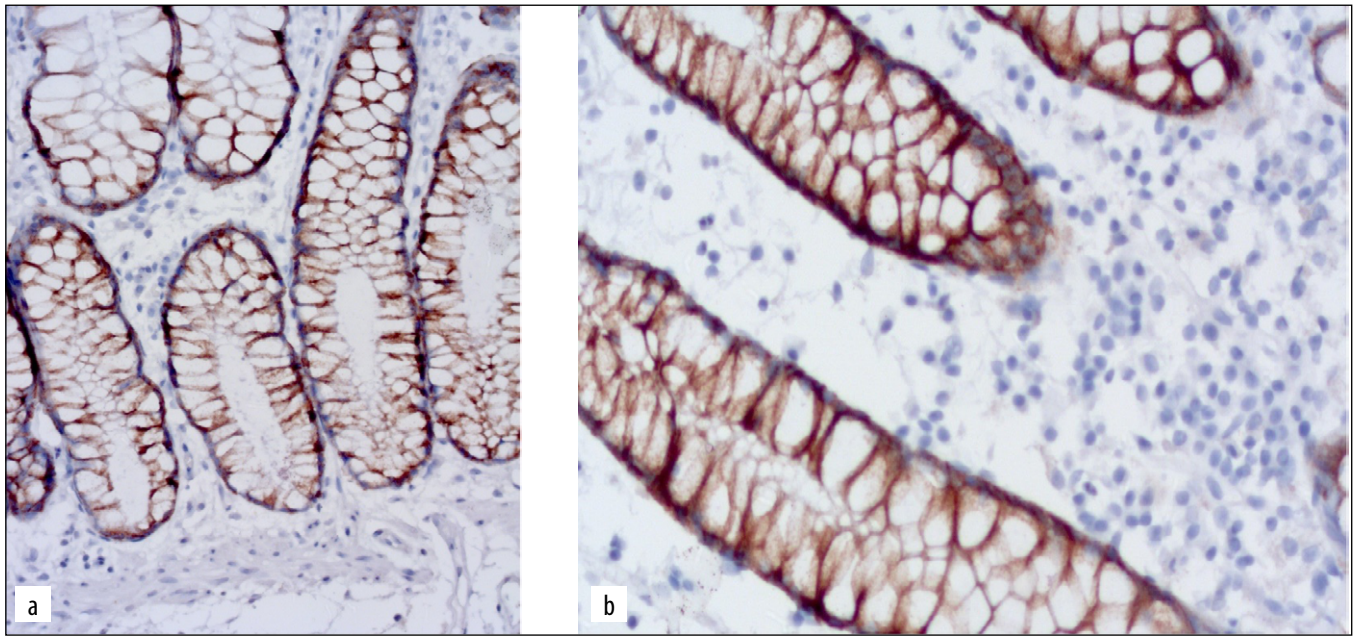

Fig. 6. Group 1. Uniform expression of MUC2 (a) and MUC4 (b) in the colon mucosa. Immunohistochemical reaction with monoclonal antibodies to MUC2 and MUC4, a) ×200, b) ×200.

plicated diverticular disease and acute uncomplicated diverticulitis were identified and analyzed. In patients with symptomatic uncomplicated diverticular disease and, especially, in patients with acute uncomplicated diverticulitis, the identified disorders were characterized by a decrease in the thickness of the mucus layer covering the surface of the colon mucosa; a decrease in the number of goblet cells, characterized by reduced size and containing several small vacuoles; decreased mucus production by goblet cells; decrease in the content of MUC2 and MUC4 in mucus. Patients with symptomatic uncomplicated diverticular disease showed a predominance of MUC4 in mucus, and patients with acute uncomplicated diverticulitis showed equal levels of MUC2 and MUC4.

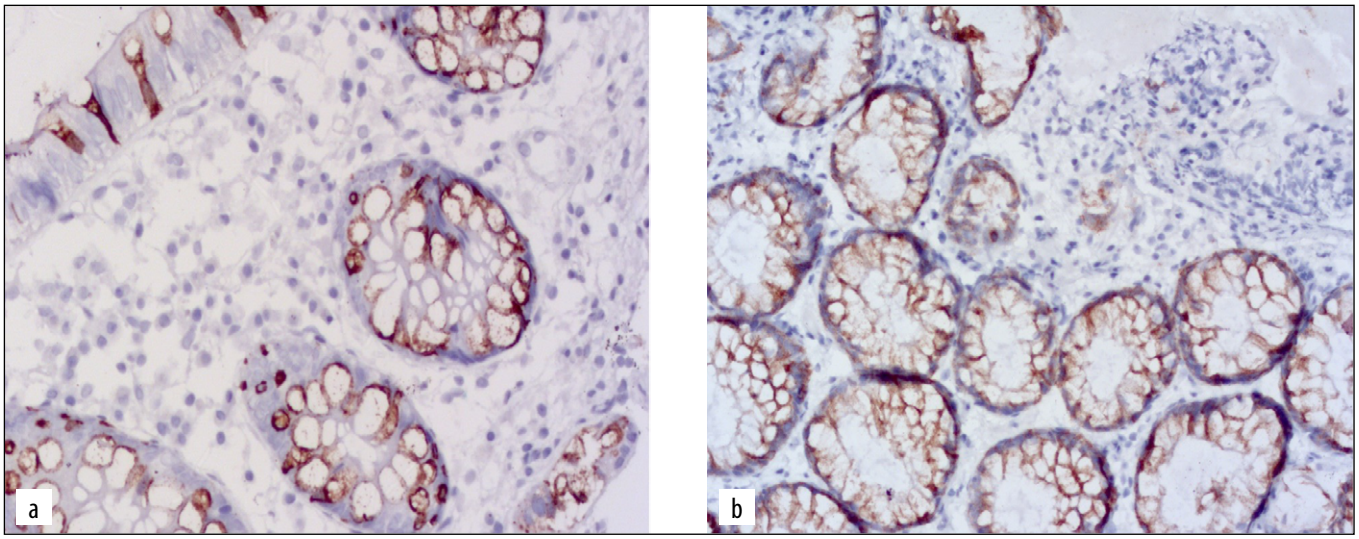

Fig. 7. Reduced and unevenly expressed expression of MUC2 (a) in group 2 and MUC4 (b) in group 3 in the colon mucosa. Immunohistochemical reaction with monoclonal antibodies to MUC2 and MUC4, a) ×200, b) ×200.

Table 1. Average brightness coefficient values in groups 1-3 in immunohistochemical reactions with monoclonal antibodies to MUC2 and MUC4

Reaction name	Group number		
	1	2	3
Reaction with monoclonal antibody to MUC2	0.42±0.007	0.49±0.006 [1]	0.57±0.009 [1,2]
Reaction with monoclonal antibody to MUC4	0.39±0.012 [3]	0.45±0.004 [1,3]	0.57±0.008 [1,2]

Note: 1 – significance of differences compared to the indicator of group 1; 2 – significance of differences compared to the indicator of group 2; 3 – significance of differences compared to the indicator in reaction with monoclonal antibody to MUC2.

The intestinal mucus gel layer is an integral structural component of the intestine used for protection, lubrication, and transport between the luminal contents and the epithelial cells [16]. The violations identified by the authors in the morphofunctional state of the mucus layer of the colon and goblet cells contained in the mucous membrane and producing mucus may underlie in the development of symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

Changes in goblet cell functions and in the chemical composition of intestinal mucus are detected in response to a broad range of luminal insults, including alterations of the normal microbiota [17]. Our previous studies revealed changes in the composition of the microbiota in the intestines in patients with symptomatic uncomplicated diverticular disease [18].

CONCLUSIONS

In patients with diverticular disease, the authors identified disturbances in the morphofunctional state of the mucus barrier of the colon, the structure and function of goblet cells contained in its mucous membrane, characterized by a decrease in the thickness of the mucus layer covering the surface of the mucous membrane; a decrease in the size and number of goblet cells with a decrease in their mucus-producing ability; a change in the mucin profile, characterized by a violation of the content of MUC2 and MUC4. These changes were greatest in patients with acute uncomplicated diverticulitis compared with patients with symptomatic uncomplicated diverticular disease. The identified disturbances in the morphofunctional state of the mucus barrier of the colon, structural and functional changes in goblet cells may be one of the mechanisms for the development of acute uncomplicated diverticulitis and symptomatic uncomplicated diverticular disease.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Mykhailo S. Myroshnychenko
Department of General and Clinical Pathological Physiology
named after D.O. Alpern, Kharkiv National Medical University,
4 Nauky avenue, Kharkiv, 61022, Ukraine
e-mail: msmyroshnychenko@ukr.net

ORCID AND CONTRIBUTIONSHIP

Hanna A. Dorohavtseva: 0000-0002-4080-5992 A D
Andrey E. Dorofieiev: 0000-0002-2631-8733 B E
Olena O. Dyadyk: 0000-0002-9912-4286 C F
Mykhailo S. Myroshnychenko: 0000-0002-6920-8374 D F

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

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ORIGINAL ARTICLE

CONTENTS

Hormonal assessment and body composition in young males with metabolic syndrome

Maria S. Cherska[2], Olena V. Maidaniuk[1], Nataliia V. Vdovenko[1], Khrystyna M. Kukharchuk[2]

[1]SCIENTIFIC RESEARCH INSTITUTE OF PHYSICAL CULTURE AND SPORT, KYIV, UKRAINE

[2]SI «V.P. KOMISARENKO INSTITUTE OF ENDOCRINOLOGY AND METABOLISM OF THE NAMS OF UKRAINE», KYIV UKRAINE

ABSTRACT

Aim: To determine the relationship between body composition and hormonal levels in young men with metabolic syndrome.

Materials and Methods: 123 males with a mean age of 24.1 ± 4.3 years (33 with metabolic syndrome (MS group) and 90 healthy physically active men (control group) were recruited at the study of body composition and hormone status. The total testosterone, cortisol, and insulin in blood serum by ELISA, the body weight (kg), lean body mass (kg) and fat mass (kg, %) by bioimpedance analysis method were investigated.

Results: It was establish the significand difference the mean value of body composition (body weight, lean body weight, fat body mass (kg, %), testosterone, cortisol insulin, and glucose concentration between MS group and control group.

Conclusions: A present study established the significant correlation of testosterone, insulin, and glucose concentration with fat body mass in all participants (MS and control groups). The negative effect of overweight (BMI > 25; FBM > 18 %) and obesity (BMI > 30; FBM > 25 %) for testosterone concentration was determined due to an increase of FBM > 20 % and insulin increasing > 9,0 μIU/l.

KEY WORDS: metabolic syndrome, testosterone, insulin, fat body mass, body mass index

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INTRODUCTION

METABOLIC SYNDROME IN YOUNG MEN

Metabolic syndrome is a group of conditions, which together increase the risk of developing atherosclerotic cardiovascular disease, insulin resistance and diabetes mellitus, and vascular and neurological diseases. Metabolic syndrome is associated with high all-cause mortality as well as mortality due to cardiovascular disease [1]. The main causes of metabolic syndrome are abdominal obesity, high blood pressure, diabetes, and dyslipidemia. With an increase in the obese population worldwide, the prevalence of metabolic syndrome is increasing [2, 3]. The global prevalence of metabolic syndrome varies slightly depending on the definition of each component and ranges from 24.3% to 45.5% [4, 5].

Since metabolic syndrome is a cluster of factors, it is difficult to manage and treat this condition compared to other diseases. The pathogenesis of metabolic syndrome can be described by a complex mechanism. Overweight and obesity are central to the development of metabolic syndrome, predisposing to hypertension, insulin resistance, and dyslipidemia, all of which are risk factors of metabolic syndrome. Physical inactivity and fatty food intake are major causes of obesity [6, 7]. There is substantial heterogeneity by sex and ethnicity in the prevalence of the metabolic syndrome [7, 8]. The syndrome is generally more common amongst young men compared with women and the prevalence tends to increase with age [9, 10].

THE SIGNIFICANCE OF FAT BODY MASS IN YOUNG MEN WITH METABOLIC SYNDROME

The fat body mass or adiposity in young men with metabolic syndrome is an important factor to consider because it is strongly associated with the development and progression of metabolic disorders. Several studies have shown that increased adiposity in young men is a significant risk factor for the development of metabolic syndrome. Excess body fat, particularly abdominal fat, is associated with insulin resistance, which can lead to high blood sugar levels and eventually type 2 diabetes. Additionally, excessive fat deposition in the liver and other organs can contribute to the development of non-alcoholic fatty liver disease (NAFLD), which is closely linked to metabolic syndrome [11-13].

Furthermore, increased adiposity is associated with chronic low-grade inflammation, which can contribute to the development of atherosclerosis and other cardiovascular diseases. This chronic inflammation can

also impair the function of the endothelium, which is the inner lining of blood vessels, leading to endothelial dysfunction and increased risk of heart attacks and strokes [14].

In conclusion, the fat body mass in young men with metabolic syndrome is a significant factor to consider as it is closely associated with the development and progression of metabolic disorders, including insulin resistance, type 2 diabetes, NAFLD, chronic inflammation, and cardiovascular diseases. Effective strategies to manage adiposity through lifestyle modifications, such as dietary changes and physical activity, can improve metabolic health outcomes and reduce the risk of cardiovascular disease in young men with metabolic syndrome [13 - 29].

INSULIN AND ITS INTERRELATIONS WITH CORTISOL AND TESTOSTERONE IN YOUNG MEN WITH METABOLIC SYNDROME

In young men with metabolic syndrome, the relationships between insulin, testosterone, and cortisol are complex and can have significant effects on their metabolic health.

Insulin resistance is a key feature of metabolic syndrome and can contribute to the development of low testosterone levels in young men. Insulin resistance leads to an increase in insulin levels, which can inhibit the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary gland. These hormones are necessary for the production of testosterone by the testes. Therefore, insulin resistance can contribute to a decrease in testosterone levels in young men with metabolic syndrome [15, 23].

On the other hand, low testosterone levels can also contribute to the development of metabolic syndrome. Testosterone has been shown to improve insulin sensitivity by increasing glucose uptake by the muscles and improving insulin signaling pathways. Therefore, low testosterone levels in young men with metabolic syndrome may lead to insulin resistance and the development of metabolic disorders [16-18].

Cortisol levels can also play a role in the relationship between insulin and testosterone in young men with metabolic syndrome. High cortisol levels have been associated with a decrease in testosterone levels in men with metabolic syndrome, as cortisol can inhibit the production of LH. Additionally, high cortisol levels can contribute to insulin resistance, as cortisol promotes the breakdown of glucose for energy, leading to an increase in blood sugar levels and a decrease in insulin sensitivity [19, 20].

It is known that metabolic syndrome at men is correlated with testosterones level. The studies (Tsai *et al.* 2000) have shown the possible association of low testosterone levels with obesity, insulin resistance and an adverse lipid profile in men. Conversely in men with metabolic syndrome and type 2 diabetes have a high prevalence of hypogonadism. Metabolic syndrome and low testosterone status are both independently associated with increased all-cause and cardiovascular mortality [21]. Men are at increased risk of developing coronary artery disease earlier in life compared with women. The reason for this bias toward males is still poorly understood. Several longitudinal studies have shown that low testosterone is an independent risk factor for the development of diabetes and metabolic syndrome [13, 22]. Baseline testosterone levels correlate inversely with the accumulation of central fat but not other fat deposits in a cohort of 110 men (Tsai *et al.* 2000). Three other studies, the Massachusetts Male Aging Study (MMAS) (Stellato *et al.* 2000), the Multiple Risk Factor Intervention Trial (MRFIT) (Haffner *et al.* 1996) and Rancho Bernado (Oh *et al.* 2002), showed an inverse correlation between baseline testosterone and the future development of diabetes. A Finnish study showed that low baseline testosterone and SHBG predicted metabolic syndrome and diabetes after a 11-year follow up (Laaksonen *et al.* 2004). The mechanisms linking testosterone with insulin resistance and type 2 diabetes are still not fully understood. Although testosterone deficiency leads to increased fat deposition and this would result in increasing insulin resistance, it may not explain the total action on insulin sensitivity. For example, one study which assessed insulin resistance by hyperinsulinaemic/euglycaemic clamps in 60 men with a range of glucose tolerance from normal to diabetic levels (Pitteloud *et al.* 2005). Their findings confirmed an inverse relation between total testosterone and insulin resistance. From muscle biopsies they showed that low testosterone impairs mitochondrial oxidative phosphorylation. As up to 70% of the body's insulin sensitivity can be accounted for by muscle this tissue may develop reduced insulin sensitivity in the hypogonadal state sufficient to contribute in part to the overall state of insulin resistance [22, 24].

Furthermore, chronic stress, which can lead to an increase in cortisol levels, is common in young men with metabolic syndrome. Chronic stress can also lead to an increase in appetite and food intake, particularly for high-calorie, high-fat foods, leading to weight gain and further exacerbating the relationship between insulin resistance, testosterone levels, and cortisol levels [19].

In conclusion, insulin, testosterone, and cortisol levels are interrelated and can influence each other in

Table 1. Instrumental and laboratory characteristics of metabolic syndrome (MS) group and control (X±б)

Indicators		MS, n=36	Control, n=91	P
TBW, kg		105,42±16,93	78,15±15,54	0,05
FBM	%	28,40±5,55	10,92 ± 4,12	0,05
	kg	29,93 ± 7,37	8,93 ± 5,38	0,05
BMI, kg/m^2		32,85± 4,42	23,75 ±3,18	0,05
LBW, kg		77,02±17,93	68,89±11,80	0,05
Glucose, mmol/l		5,31±0,92	4,54 ±0,43	0,05
HOMA-IR index		3,18±1,59	0,99 ±0,79	0,05

Note: TBW - total body weight; FBM - fat body mass, BMI – body mass index; LBW – lean body weight; HOMA-IR - homeostatic model assessment of insulin resistance. $p< 0.05$ – statistically significant differences.

Table 2. Hormonal status of metabolic syndrome (MS) group and control (X±б)

Indicators	MS, n=36	Control, n=91	P
Testosterone, nmol/l	15,29±4,83	25,13±11,13	0,05
Cortisol, nmol/l	303,24±76,41	638,41±317,99	0,05
Insulin, μIU/l	13,27±6,26	5,52±3,77	0,05

Note: the average value ± SD is given for the case of the normal distribution law. $p< 0.05$ – statistically significant differences.

complex ways in young men with metabolic syndrome. Lifestyle modifications, such as weight loss, regular exercise, acute influence of physical loads for hormone content [25-26] and a healthy diet, can help improve insulin sensitivity and testosterone levels while reducing cortisol levels and overall stress.

AIM

The aim of this study was to determine the relationship between body composition and hormonal levels in young men with metabolic syndrome.

MATERIALS AND METHODS

In line with the study's objectives, we enrolled 123 male participants with an average age of 24.1 ± 4.3 years. This cohort consisted of 33 men with both metabolic syndrome and a sedentary lifestyle (referred to as the «MS group» with a mean age of 23.3 ± 4.8 years) and 90 healthy, physically active men (referred to as the «control group» with a mean age of 24.4 ± 4.6 years). The individuals in the control group engaged in an average of 4 hours of physical activity per day, six days a week, resulting in an average of 24.87 ± 2.1 hours of weekly physical activity. Our study included patients who were examined at the consultative and diagnostic departments of the "V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine" and the Scientific Research Institute of Physical Culture and Sport. Before undergoing examination, all patients were informed about the research protocol, which adhered to the ethical principles outlined in the Helsinki Declaration of the World Medical Association, the Council of Europe's Convention on human rights and biomedicine, and the legislation of Ukraine. They provided their informed consent for participation in the study and did not receive any medications.

To identify signs of metabolic syndrome in the participants, we utilized the IDF criteria from 2005. In our controlled clinical study of metabolic syndrome patients, we employed elements of typological sampling (stratification randomization). Inclusion criteria: male gender, age 18-45 years old, absence of any earlier determined diseases and prescribed medications. All patients underwent standard clinical and laboratory assessments, including hormonal blood parameters (total testosterone, cortisol, and insulin). Total body weight (TBW, kg), fat body mass (FBM, kg, %), lean body weight (LBW, kg) were determined using bioelectric impedance analysis with InBody 770 from the Republic of Korea. The HOMA IR indicator was calculated using the formula: HOMA IR = fasting blood glucose (mmol/l) × fasting blood insulin (μU/l) / 22.5 [Matthew D. R., 1985]. We assessed the degree of general obesity using BMI indicators in accordance with the recommendations of the WHO (1997) and the International Diabetes Federation (2005). A BMI within the normal range was defined as less than 24 kg/m^2, and waist circumference (WC) was considered normal if it was less than 94 cm in men. The HOMA IR indicator, which should not exceed 2.77, was also used for evaluation. To determine the concentration of testosterone, cortisol, and insulin in blood serum, we

Table 3. Body composition and biochemical parameters at men with different range of BMI (X±б)

Indicators	MS, n=33		Control, n=91		
	OB, n=23	OW, n=10	OB, n=5	OW, n=22	NW, n=65
TBW, kg	109,50±18,30	96,00±7,50*	120,20±15,54a	94,40±10,00#q	70,80±8,30
FBM, %	28,20±5,60	28,90 ± 5,80	19,80 ± 7,80a	13,40 ± 3,60#q	9,60 ± 2,80
FBM, kg	30,90 ± 8,00	27,70 ± 5,50	24,10 ± 10,00a	12,80 ± 3,50#q	6,80 ± 2,40
BMI, kg/m^2	34,60± 4,20	28,80 ±1,20*	33,30 ±2,20a	27,20 ± 1,50#q	22,20 ± 2,40
LBW,kg	81,40±9,00	67,10±10,00 *	96,20±8,20 a	80,90±7,40#q	63,60±7,40
Glucose, mmol/l	5,26±0,99	5,42 ±0,78	4,82 ±0,41	4,63 ±0,48	4,49 ±0,41
HOMA-IR index	3,10±1,71	3,50±1,29	1,80 ±0,97a	1,10 ±0,62#	0,90 ±0,78
Testosterone, nmol/l	16,20±5,10	13,30±3,60	21,60±5,80	23,60±11,20	25,70±11,30
Cortisol, nmol/l	301,20±83,20	308,00 ± 61,60	541,30 ± 192,70	716,70 ± 318,20	621,30 ± 322,90
Insulin, μlU/l	12,80 ± 6,70	14,40 ± 5,20	8,60 ± 4,40a	6,70 ± 3,90q	5,00 ± 3,60

Note: the average value ± SD is given for the case of the normal distribution law;
* - statistically significant differences (p< 0.05) between obese and overweight group in men with metabolic syndrome;
a - statistically significant differences (p< 0.05) between obese and normal weight men in control group;
- statistically significant differences (p< 0.05) between obese and overweight men in control group;
q - statistically significant differences (p< 0.05) between overweight and normal weight men in control group.

employed ChemWell enzyme-linked immunosorbent assay equipment from Awareness Technology (USA) with the use of AccuBind ELISA test systems from Monobind Inc. (USA). Blood sampling and subsequent processing followed the provided instructions. Our study excluded individuals with stable heart rhythm disorders (such as atrial fibrillation or frequent ventricular extrasystoles), clinically diagnosed heart failure, severe kidney or liver dysfunction, drug or alcohol addiction, and those who had experienced acute inflammatory diseases within the previous month.

All statistical analyses were performed using «STATISTICA 12» with a significance level set at 0.05 unless stated to the contrary. One-way ANOVA, correlation analysis (Spearman correlation) was used for determine the significant of the hormone status for BMI and percent body fat in young men with metabolic syndrome and physically active healthy. The Dunkan method (post hoc analysis) was used for differences estimation between control and MS groups. The correspondence of the sample to the normal distribution was checked for asymmetries and excesses, which indicated the proximity of the distribution to the normal curve.

RESULTS

Firstly, patients were divided into 2 groups: 1 – young men with metabolic syndrome and 2- control one (physically active and healthy individuals). Then we compared the patients' main instrumental and laboratory characteristics (Table 1). The differences in hormonal indicators are demonstrated in table 2.

We have established statistically significant differences in all instrumental and laboratory parameters of the studied groups. So, total body weight (TBW, kg) of control group was 25.71% less; fat body mass (FBM, %) was 61.65% less and insulin levels were 58.40% lower than in men with metabolic syndrome, while cortisol and testosterone levels were 52.51% and 40.00% higher.

Thus, we have found significant differences in all characteristics of two groups. Secondly, to consider detailed differences between groups, we decided to divide each group into three sub-groups depending on their BMI into three main groups: normal weight men with BMI = 18,5-24,9 kg/m^2 (NW), overweight ones with BMI= 25,0 – 29,9 kg/m^2 (OW) and obesity ones with BMI above 30,0 kg/m^2 (OB) (Table 3).

The following results were obtained. Firstly, in the group with metabolic syndrome, there was a statistically significant difference only in terms of TBW and LBW, and in the obese group this indicator was 17.28% higher than in the overweight group. The FBM and hormones level in metabolic syndrome OW and OB groups don't differ (Table 3).

Secondly, in the control group, statistically significant differences in body composition assessment indicators were established both between groups with obesity and overweight, and when comparing these groups with normal body weight.

In the men of control group with BMI > 30 (OB subgroup), FBM (%) was 51.52% and 32.32% higher than

Table 4. Body composition and biochemical parameters at men with different range of BMI and FBM

Indicators		MS, n=33		Control, n=91		
		OB, n=12	OW, n=21	OB, n=2	OW, n=9	NW, n=80
TBW, kg		102,80±13,40	106,90±18,80	124,10±15,54[a]	100,90±13,70[#q]	75,20±12,50
FBM	%	34,00±3,80	25,20 ± 3,40[*]	27,10 ± 1,80[a]	17,90 ± 2,00[#q]	9,80 ± 2,60
	kg	34,90 ± 5,70	27,10 ± 6,70[*]	33,60 ± 2,00[a]	18,10 ± 3,50[#q]	7,40 ± 2,70
BMI, kg/m^2		31,70± 3,70	33,50 ±4,80	35,50 ±0,20[a]	28,30 ± 2,40[#q]	23,10 ± 2,40
LBW, kg		68,80±14,30	81,70±14,63[*]	90,50±11,80[a]	82,80±10,90[q]	67,00±10,50
Glucose, mmol/l		5,70±1,00	5,10 ±0,80	4,60 ±0,20	4,80 ±0,40[q]	4,50 ±0,40
HOMA-IR index		3,90±1,60	2,80±1,40[*]	1,50 ±1,30	1,50 ±0,90[q]	0,90 ±0,70
Testosterone, nmol/l		13,00±2,70	16,60±5,30[*]	21,10±15,54	24,50±12,30	25,20±11,10
Cortisol, nmol/l		288,70±61,20	311,60 ± 84,20	495,60 ± 200,00	703,50 ± 307,30	636,60 ± 321,40
Insulin, μlU/l		15,50 ± 6,70	12,00 ± 5,80	7,20 ± 6,20	6,50 ± 4,00	5,40 ± 3,70

Note: the average value ± SD is given for the case of the normal distribution law.
* - statistically significant differences ($p< 0.05$) between obese and overweight group in men with metabolic syndrome;
a - statistically significant differences ($p< 0.05$) between obese and normal weight men in control group;
- statistically significant differences ($p< 0.05$) between obese and overweight men in control group;
q - statistically significant differences ($p< 0.05$) between overweight and normal weight men in control group.

that of men with normal and overweight, and LBW - by 33.89% and 15.90%, respectively.

It should be noted that the HOMA-IR index in the group of men with metabolic syndrome exceeded normal values (normal up to 2.7), while in the control group it was within the normal range. However, a statistically significant difference was obtained in the HOMA-IR index between control subgroups with obesity compared to overweight and normal body weight by 38.89% and 50.00%, respectively, against the background of a statistically significant difference in insulin levels established in these subgroups - by 22.09% and 41.86% respectively.

Thirdly, we decided to divide each group into three sub-groups depending on their BMI + FBM into three main groups: normal weight men with BMI= 18,5-24,9 kg/m^2 and FBM ≤ 18 %, overweight ones with BMI= 25,0 – 29,9 kg/m^2, FBM 18,1 - 25 % and obesity ones with BMI above 30,0 kg/m^2, FBM above 25 % (Table 4).

Based on the analysis, we found that there was no statistically significant difference in the levels of insulin and cortisol in both groups. Testosterone rates in men with metabolic syndrome were statistically significantly higher at 21.69% in the overweight group than in obese men.

To determine the relationship between body composition and hormonal levels, a correlation analysis was carried out in each group separately and in the total sample. Thus, in the group of men with metabolic syndrome, BMI had a direct correlation only with testosterone concentration (r=0.46), while FBM has an inverse correlation with testosterone (r=-0.36) and a direct correlation with insulin (r=0,47). In the control group low correlation was found between FBM and hormone levels, in BMI it had a direct correlation with insulin (r=0.22).

DISCUSSION

First and foremost, our study draws attention to the difference in hormone levels (insulin, cortisol, testosterone) and BMI and FBM in two comparative groups of young men. This once again underscores the contribution of regular physical activity to hormonal health in individuals, even at a young age.

The obtained results from comparing various body composition indicators based on BMI emphasize once again the need for a more extensive and thorough analysis of these indicators (FBM, TBW, LBW) in patients with varying body weights. In alignment with many other contemporary studies, our research demonstrates the lack of universality of a metric such as BMI in assessing metabolic health [1, 4 - 6].

The absence of an increase in the HOMA-IR index in the group of young men with metabolic syndrome and the presence of a statistically significant difference in the HOMA-IR index between control groups with obesity compared to overweight and normal body weight by 38.89% and 50.00% is very interesting and discussable question. Several factors can potentially explain these findings: 1. The control group men in the study might have significantly improved insulin sensitivity due to regular exercise, which can counteract the effects of obesity or overweight to some extent. Regular physical activity can enhance glucose uptake by muscles and

improve insulin sensitivity. 2. The control group may follow a specific diet that promotes better insulin sensitivity. Their dietary habits could be a contributing factor to maintaining lower HOMA-IR values despite obesity or overweight. 3. Genetic factors can also play a role. Some individuals may have genetic predispositions that make them less susceptible to insulin resistance, even in the presence of excess body weight. 4. The control groups' hormonal profile, including factors such as testosterone and adiponectin, could influence insulin sensitivity. Their hormonal balance may be more favorable compared to the group with metabolic syndrome. 5. The composition of the weight (lean muscle mass vs. fat mass) can be different between the groups. Control group' men may have a higher proportion of lean muscle mass, which can improve insulin sensitivity.

In common, metabolic syndrome is a complex condition with varying degrees of severity and individual factors. It's possible that the metabolic syndrome group in this study represents a milder form of the condition. Understanding the exact reasons for these findings would likely require further investigation, possibly through additional studies and more comprehensive assessments of the participants' lifestyles, genetics, and metabolic health. The study's sample size and how individuals were selected for each group can also impact the results.

FBM and testosterone levels are closely linked, and there is a bidirectional relationship between them. Testosterone is an anabolic hormone that plays a crucial role in regulating body composition, including fat mass and lean mass. Studies have shown that increased FBM is associated with lower testosterone levels, and vice versa [21]. Adipose tissue, particularly visceral fat, contains an enzyme called aromatase, which converts testosterone to estrogen, leading to a decrease in testosterone levels. Furthermore, the relationship between FBM and testosterone levels is particularly relevant in men with metabolic syndrome. Metabolic syndrome is associated with an increased risk of low testosterone levels, which can exacerbate metabolic dysfunction, including insulin resistance and dyslipidemia. In our study we also found direct correlation of BMI and inverse one of FBM with testosterone concentration (r=0.46 and r=-0.36 respectively) in metabolic syndrome young men. Our results are consistent with data of other authors [21-24], but unlike other studies, our research exclusively involved young men (under 44 years old), emphasizing both the early onset of testosterone level reduction and the independence of this issue from age.

FBM and insulin are also closely linked, and excess adiposity is a major risk factor for insulin resistance and type 2 diabetes: Excess adiposity, particularly visceral fat, leads to the release of pro-inflammatory cytokines, which can impair insulin signaling and contribute to insulin resistance [28, 30, 31]. In our study we found the strong correlation between FBM and insulin level (r=0,47) only in metabolic syndrome group. This further underscores the strong association between excess weight and visceral obesity with the development of type 2 diabetes, as well as the significance of regular physical activity even in young individuals. What makes our study unique is that we did not find correlations between the BMI and insulin levels, only with FBM, whereas in most other studies, authors examined such associations primarily with the BMI [7, 9, 15, 27].

CONCLUSIONS

A present study established the significant correlation of testosterone, insulin, and glucose concentration with FBM in all participants (MS and control groups). The negative effect of overweight (BMI > 25; FBM > 18 %) and obesity (BMI > 30; FBM > 25 %) for testosterone concentration was determined due to an increase of FBM > 20 % and insulin increasing > 9,0 μIU/l. Low testosterone and high insulin levels are associated with an increased risk of cardiovascular disease. Early effective strategies to manage adiposity and improve metabolic health outcomes especially in young men, including dietary changes and physical activity, can help improve testosterone and insulin levels and reduce the risk of cardiovascular diseases.

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CONFLICT OF INTEREST
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CORRESPONDING AUTHOR
Maria S. Cherska
SI «V.P. Komisarenko Institute of Endocrinology
and Metabolism
of the NAMS of Ukraine»
69 Vyshgorodska st., 04114 Kyiv, Ukraine
e-mail: emariya83@gmail.com

ORCID AND CONTRIBUTIONSHIP
Maria S. Cherska: 0000-0002-3689-2683 A D E F
Olena V. Maidaniuk: 0000-0003-0451-1847 A C D E F
Nataliia V. Vdovenko: 0000-0002-3097-5920 B E F
Khrystyna M. Kukharchuk: 0000-0002-2495-2693 B D
Olha A. Haiova: 0000-0003-1309-1899 D

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

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ORIGINAL ARTICLE

CONTENTS

Characteristics of the hormonal background in women with abnormal uterine bleeding and extragenital disorders

Liudmyla A. Vygivska, Roman Y. Blahoveshchenskyi, Yevgenii V. Blahoveshchenskyi, Yevgeniia B. Radzischevska, Iryna A. Guz, Olena V. Buhaiova, Olesia O. Pliekhova
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Aim: To establish the peculiarities of the hormonal background in women with abnormal uterine bleeding and extragenital disorders.

Materials and Methods: The study involved examination of 100 women of reproductive age with concomitant ED and AUB (MG). CG included 50 healthy women. MG women were additionally divided into subgroups depending on the detected pathological changes in the uterine cavity. When examining the hormonal status, concentrations of FSH, LH, estradiol, progesterone, and leptin in BS were determined. Additionally, the level of 25-hydroxyvitamin D ((25-OH) D) was determined.

Results: The decrease in FSH level in subgroups of GP, PSL, EH, EHL was 1.8-2.4 times (KWT, $p<0.01$). LH in BS of MG patients was statistically lower than that of CG patients (MWT, $p=0.0083$). The lowest level of LH was registered in patients with EHL, which was 2 times lower than this indicator in CG. A statistically significant increase in the level of estradiol was registered in 73% of MG patients (MWT, $p=0.044$). The lowest level of progesterone was registered in patients with EHL - 8.40, which is 4.7 times lower than in CG (MWT, $p=0.0021$). A statistically significant increase in the level of leptin in BS was observed in MG patients (KWT, $p=0.0021$). The highest level of leptin was found in women with AFP, 2.3 times higher than CG indicators. A statistically significant correlation between the level of leptin and BMI ($r=0.86$, $p=0.011$) and a statistically significant ($p=0.023$) correlation between the level of leptin and estradiol in BS of patients of the examined groups ($r=0.42$) were revealed. In 87% of MG women, vitamin D deficiency was observed in BS (KWT, $p=0.03$). A statistically significant ($p=0.01$) negative correlation between the level of estradiol and vitamin D in the BS of female patients was revealed ($r=-0.61$, $p=0.01$).

Conclusions: Women of reproductive age with AUB and ED were found to have disorders in the hypothalamic-pituitary-ovarian system. Most patients are characterized by an elevated level of leptin, the concentration of which is closely correlated with BMI, and an elevated level of estradiol is correlated with the level of vitamin D.

KEY WORDS: abnormal uterine bleeding, extragenital disorders, hormonal status, vitamin D level

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INTRODUCTIONS

The problem of preserving the reproductive health of women of reproductive age is relevant both throughout the world and in Ukraine, which is associated with a significant increase in the level of gynecological morbidity in this population of women [1].

AUB is a debilitating symptom that affects up to one third of women of reproductive age [2]. According to foreign researchers [3], this condition is the most common disorder in women of reproductive age, the fourth most common reason for referral to gynecological services. Approximately 20% of 1.2 million visits to specialized gynecological services are related to this disorder [4].

AUB most often does not threaten life, but significantly reduces its quality [5]. It is reported that throughout the world the prevalence of AUB is observed within fairly wide limits from 3 to 52%. Such a discrepancy can be explained by a slightly different interpretation of the specified disorder and evaluation system: subjective or objective [4,6]. Subjective assessment is most likely to show a higher prevalence of AUB versus objective assessment, as it is based on the woman's personal perception of bleeding. Studies have found that women with AUB score below the 25th percentile on health-related quality of life scores when compared to the general female population of the same age [7]. Menstrual cycle impairments affect

all aspects of a woman's life, even increasing the risk of premature death [8]. Women with AUB are more likely to seek medical care due to social and physical factors [9], while 50% of female patients have mental health problems due to anxiety and depression [10].

Structural causes of uterine bleeding increase with increasing reproductive age and can affect reproductive health. Women in high-income countries on average decide to postpone childbirth [11]. Therefore, structural changes of the myometrium, such as uterine myoma, can have a negative impact on reproductive outcomes, reduce the probability of natural conception and reduce the success of assisted reproductive methods, even contributing to pregnancy complications [12].

AUB can also be caused by extragenital disorders (ED). This is explained by the features of the endometrium, which significantly depends on changes in the general hormonal status of a woman, which is largely influenced by the functional state of some organs and systems [13]. AUB can result from thyroid dysfunction, uncontrolled diabetes, obesity, blood coagulation disorders (most often von Willebrand's disease), infectious diseases, taking certain medications (corticosteroids, antipsychotics or antiepileptics), climate change and nutrition, psychological stress, etc. [14].

Individuals with this disorder often form a group, which leads to significant problems with the choice of individual treatment. Therefore, scientific research on the assessment of hormonal status, vitamin D level will allow to identify a certain group of patients in accordance with the principles of personalized medicine and will contribute to more effective treatment. Solving these problems will help improve the quality of life of women and avoid invasive surgical treatment.

AIM

To establish the peculiarities of the hormonal background in women with abnormal uterine bleeding and extragenital disorders.

MATERIALS AND METHODS

The research was conducted in the Communal non-profit enterprise "Kharkiv Maternity Hospital No. 1" of Kharkiv City Council. The study involved 100 women of reproductive age with accompanying extragenital disorders, who sought help for AUB and made up the main group (MG). Control group (CG) included 50 women of reproductive age who visited the clinic for a prophylactic examination. The most common extragenital disorder were circulatory, endocrine and digestive diseases. Moreover, 12 patients (12%) were simultaneously diagnosed with hypertension and obesity, 5 (5%) with obesity and varicose veins, 11 (11%) with neurocirculatory dystonia and chronic gastritis, and 10 (10%) with obesity and diabetes, 3 (3%) had chronic gastritis and obesity [15]. MG women were further divided into subgroups depending on the detected pathological changes in the uterine cavity: endometrial polyp (EP) (glandular polyp (GP), adenofibromatous polyp (AFP), submucous leiomyoma (SL), endometrial polyp and submucous leiomyoma (PSL), endometrial hyperplasia (EH), endometrial hyperplasia in combination with submucosal leiomyoma (ESL) [16].

To determine the state of the endocrine function of the pituitary gland and ovaries, the level of gonadotropic and steroid hormones in blood plasma was established. When examining the hormonal status of the women of the studied groups, concentrations of LH, FSH, progesterone, estradiol in blood serum (BC) were determined by enzyme-linked immunosorbent assay (ELISA) using standard "Biointernation" test kits, France. Leptin was determined in BS by solid-phase ELISA, NTI Immunochem 2100 analyzer, using DRG Leptin ELISA Kits. 25-hydroxyvitamin D ((25-OH)D) was determined in BS by the competitive ELISA method using a luminescent reagent (Siemens Healthcare Diagnostics (Germany) on an Atellica IM 1600 analyzer (Germany).

Methods of statistical analysis. For statistical data processing, the general-purpose data processing software package "Statistica for Windows" was used.

Median and mean values were employed to present the data as measures of location; standard deviation and quartiles as measures of dispersion; minimum and maximum value as indicators of sample size.

The Kruskel-Wallis test was used as a non-parametric method of comparing independent groups, the Mann-Whitney test was used to compare two independent groups, and the Spearman correlation coefficient was used to assess the closeness of the relationship between indicators.

RESULTS

Conducting an exploratory analysis of a group of variables using the Kraskel-Wallis rank test (KWT) demonstrated the presence of a statistically significant dependence on the group ($p<0.05$) of all the indicators used. That is, the analysis of indicators in groups is appropriate and potentially informative.

Table 1. FSH level in women with AUB and ED, (mIU/ml)

Clinical groups	Statistical indicators						
	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
GP	3.81	3.20	1.80	7.40	2.00	5.40	2.12
AFP	5.50	6.1	2.90	7.40	3.2	7.3	1.82
SL	4.88	5.30	2.00	6.40	4.30	5.90	1.37
PSL	3.30	3.00	1.90	7.10	2.70	3.50	1.24
EH	3.41	3.10	1.90	6.30	2.70	3.80	1.17
ESL	3.83	3.80	1.80	6.40	2.90	4.20	1.16
MG	7.35	7.15	3.60	10.70	6.40	8.40	1.54

Table 2. LH level in women with AUB and ED, (mIU/ml)

Clinical groups	Statistical indicators						
	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
GP	3.61	3.00	1.90	6.30	2.70	4.90	1.43
AFP	3.34	3.70	1.90	4.20	2.00	4.20	0.97
SL	3.65	3.70	2.10	5.20	2.90	4.30	1.02
PSL	3.53	3.50	1.90	5.30	2.70	4.20	1.01
EH	4.10	3.70	2.20	6.30	3.10	5.20	1.18
ESL	3.35	2.95	1.90	6.10	2.50	3.80	1.24
MG	5.83	5.95	2.10	7.90	5.20	6.70	1.44

Data on the level of FSH in the BS of women of the main and control groups are shown in Table 1.

From these data, it can be seen that the characteristics of the central tendency and range of blood FSH levels of MG patients are generally lower than those of CG patients. The decrease in the level of this hormone in the subgroups of GP, PSL, EH, ESL was 1.8-2.4 times and had a statistically significant character (KWT, $p<0.01$), while in the subgroup of patients with AFP and SL there is a tendency to its increase to normal.

Data on the level of LH in the BS of women of the main and control groups are shown in Table 2.

From the given data, it can be seen that LH in the BS of MG patients is statistically lower than that of patients in the control group (Mann-Whitney test (MWT), $p=0.0083$). Thus, the lowest level of this hormone occurred in patients with SL and was 2 times lower than this indicator in CG.

Data on the level of estradiol in the BS of women of the main and control groups are shown in Table 3.

A statistically significant increase in the level of estradiol was registered in 73% of MG patients (MWT, $p=0.044$). The median value, as well as the levels of the minimum and maximum levels of this hormone in BS exceeded this indicator in the control group by 2.7-3.8 times.

Table 3. Estradiol level in women with AUB and ED, (pg/ml)

Clinical groups	Statistical indicators						
	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
GP	345.98	385.40	200.30	400.50	296.30	397.90	68.56
AFP	282.37	275.40	223.10	390.70	226.40	345.90	64.87
SL	365.96	364.90	321.10	396.50	352.10	378.30	22.81
PSL	376.02	385.20	278.80	400.50	363.20	395.40	30.51
EH	297.71	289.60	214.90	399.50	256.40	344.80	56.17
ESL	316.05	311.30	211.80	405.30	276.80	368.40	60.23
MG	94.97	99.10	28.90	150.30	58.20	124.80	38.96

Table 4. Progesterone level in women with AUB and ED, (nmol/l)

Clinical groups	Statistical indicators						
	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
GP	11.22	10.10	7.30	19.70	8.30	13.20	3.98
AFP	14.45	14.90	12.50	16.10	13.70	15.30	1.19
SL	16.84	17.50	12.50	19.20	14.60	18.90	2.50
PSL	14.40	15.10	9.70	17.20	12.70	16.30	2.24
EH	13.02	12.60	7.90	18.60	10.50	16.00	3.19
ESL	10.39	8.40	7.30	19.60	7.90	11.90	3.172
MG	47.21	39.75	15.60	90.10	29.50	64.50	22.53

Data on the level of progesterone in the BS of women of the main and control groups are shown in Table 4.

When examining the level of the hormone progesterone in the BS of MG patients, there is a statistically significant decrease in its level compared to the control group (MWT, p=0.0021). The lowest level of this hormone was registered in patients with ESL - 8.40, which is 4.7 times lower than in CG and in patients with GP - 10.10, which is 3.9 times lower than in CG. The highest level of progesterone is noted in patients with SL - 17.50, which is twice the level of this hormone in patients with ESL and 2.2 times lower than the level in CG.

Data on the level of leptin in the BS of women of the main and control groups are shown in Table 5.

A statistically significant increase in the level of leptin in BS was observed in MG patients (KWT, p=0.0021). The highest level of leptin is observed in women with AFP, 2.3 times higher than the control group.

The analysis of relationships revealed a statistically significant correlation between leptin level and BMI (r=0.86, p=0.011). In addition, a statistically significant (p=0.023) correlation was found between leptin and estradiol levels in the BS of patients of the examined groups (r=0.42).

Data on the level of vitamin D in the BS of women of the main and control groups are shown in Table 6.

Table 5. Leptin level in women with AUB and ED, (ng/ml)

Clinical groups	Statistical indicators						
	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
GP	12.64	13.01	7.00	19.70	8.20	15.30	4.27
AFP	14.55	16.03	8.06	19.70	9.72	19.61	5.1
SL	11.71	9.06	6.80	18.04	7.60	16.05	4.83
PSL	12.51	13.65	6.30	18.09	8.65	16.05	3.93
EH	11.79	13.01	6.50	17.08	7.90	15.07	3.87
ESL	11.50	10.87	7.02	17.03	8.67	14.05	3.37
MG	7.88	6.78	3.63	15.07	5.07	10.06	3.42

Table 6. Vitamin D level in women with AUB and ED, (ng/ml)

Clinical groups	Statistical indicators						
	Average	Median	Minimum	Maximum	Lower quartile	Upper quartile	Standard deviation
GP	16.27	11.70	6.80	33.40	9.00	24.80	9.37
AFP	16.27	20.40	5.40	27.20	6.40	23.10	8.79
SL	23.54	19.43	11.00	41.07	13.80	37.80	12.42
PSL	15.70	10.50	6.35	32.80	8.60	23.80	9.51
EH	21.34	17.30	5.90	40.40	12.30	31.50	10.78
ESL	19.48	19.60	7.30	38.90	9.70	26.30	9.71
MG	39.85	37.85	26.70	70.40	32.80	43.10	9.56

In 87% of MG women, vitamin D deficiency was observed in BS, that is, its level was lower than 20 ng/ml, and in 13% insufficiency of this vitamin (20-29 ng/ml) (WMT, p=0.03). During the study, a statistically significant (p=0.01) negative correlation was found between the level of estradiol and vitamin D in the BS of patients of the examined groups (r= -0.61, p=0.01).

The conducted study showed that changes in the hormonal background were observed in patients with AUB and ED.

DISCUSSION

AUB refers to any irregular or unusual uterine bleeding that is not part of a woman's normal menstrual cycle. It can also indicate serious health problems. The modern theory of the pathogenesis of hyperplastic processes of the endometrium is based on the mechanisms of its estrogen stimulation. The main regulatory hormones in the endometrium are estrogens and progesterone, which can act directly or indirectly [17,18]. MG patients, on the background of hyperestrogeny, were

found to have insufficiency of the 2nd phase of the menstrual cycle.

The conducted research showed that changes in the hormonal background were observed in patients with AUB and ED. A decrease in the levels of FSH, LH, and progesterone was noted against the background of hyperestrogenemia and hyperleptinemia. The lowest levels of these hormones were registered in patients with PSL (FSH - 3.00 mIU/ml), with ESL (LH - 2.95 mIU/ml, progesterone - 8.40 nmol/l). The highest levels of estradiol (385.4 pg/ml) were determined in patients with GP. An elevated level of leptin was characteristic of most MG women (the highest in patients with AFP - 16.03 ng/ml), and its concentration was closely correlated with BMI (r=0.86, p=0.0192). In 87% of MG women, vitamin D deficiency was observed in BS, and in 13% insufficiency of this vitamin. Correlational dependences were determined between the level of leptin and estradiol (r=0.42, p=0.0021), estradiol and vitamin D (r= -0.61, p=0.044).

Therefore, leptin levels increased in MG women. The determined correlation between the level of leptin and estradiol, estradiol and vitamin D indicated that the examined women had a metabolic syndrome with additional synthesis of estrogens in adipose tissue and vitamin D deficiency in BS.

In the last decade, more and more literary data are found, which say that adipose tissue is one of the most important endocrine organs. [19,20] Adipose tissue is capable of producing many hormone-like substances, including leptin [21]. The main functions of these substances are the regulation of eating behavior, energy metabolism, control of the functional activity of the cardiovascular system, regulation of the processes of oncogenesis and metastasis [22,23]. Currently, adipose tissue cells are considered as endogenous regulators and modulators of reproductive system functions [24,25].

According to Lebrethon M. C. et al., leptin is a "tissue hormone" that takes part in the regulation of appetite, secretion of gonadotropin-releasing hormones (GRH) by the hypothalamus and gonadotropins by the pituitary gland. Leptin affects the sensitivity of gonadotrophs to GRH, stimulates the release of LH and FSH by gonadotrophs [26]. J. Kitawaki et al. obtained data on the stimulating effect of leptin on the synthesis of estrogens directly through the activation of aromatase in granulosa cells [27].

Vitamin D deficiency has become a problem today due to its increased prevalence among women of reproductive age [28]. Recently, much attention has been paid to the study of the relationship between vitamin D and the characteristics of the menstrual cycle of women. [29]. Thus, a study by K. Łagowska [30] demonstrated the relationship between the frequency of menstrual disorders and low levels of vitamin D in women. Vitamin D controls estrogen biosynthesis and directly regulates the aromatase gene [31].

CONCLUSIONS

In women of reproductive age with AUB and ED, there are impairments in the hypothalamic-pituitary-ovarian system: a decrease in the levels of FSH, LH, progesterone against the background of hyperestrogenemia, hyperleptinemia, and a reduced level of vitamin D. Most patients are characterized by an increased level of leptin, the concentration of which is closely correlated with BMI , and the increased level of estradiol correlates with the level of vitamin D. So, menstrual function is to some extent related to the content of leptin and its relationship with estrogens and the level of vitamin D in blood serum, which is an important link in the pathogenesis of AUB against the background of ED.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Liudmyla A. Vygivska
Kharkiv National Medical University, Kharkiv, Ukraine
4 Nauky avenue, Kharkiv, 61022, Ukraine
e-mail: liudmilavygovskaya@gmail.com

ORCID AND CONTRIBUTIONSHIP
Liudmyla A. Vygivska: 0000-0002-9389-4845 A D F
Roman Y. Blahoveshchenskyi: 0000-0003-3022-8180 B F
Yevgenii V. Blahoveshchenskyi: 0000-0002-0735-6515 B F
Yevgeniia B. Radzischevska: 0000-0001-9149-7689 C
Iryna A. Guz: 0000-0002-6785-4903 B F
Olena V. Buhaiova: 0009-0003-7096-9335 A F
Olesia O. Pliekhova: 0000-0003-3183-8183 A F

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ORIGINAL ARTICLE

CONTENTS

Influence of different factors (duration of disease, gender, education, patients' history, job and age) in metformin response in type 2 diabetes mellitus patient

Alaa Abd AL-Hussain Naem[1], Mona N Al-Terehi[2], Fadhaa Abdulameer Ghafil[1], Sahar Majeed[1], Najah Rayish Hadi[1], Defaf Al-Mudafer[3]

[1]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ.
[2]DEPARTMENT OF MOLECULAR BIOLOGY, FACULTY OF SCIENCE, UNIVERSITY OF BABYLON, BABIL, IRAQ.
[3]DEPARTMENT OF MICROBIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ.

ABSTRACT

Aim: This study aims to evaluate how various factors affect various aspects of glycemic control in individuals with type 2 diabetes who are undergoing metformin treatment.

Materials and Methods: A cross-sectional study involved 150 participants who met specific criteria, including being aged between 30 and 70, having a type 2 diabetes diagnosis, and using 1000 mg of metformin as the monotherapy for at least three months. Collected data encompassed various measures, such as levels of glycated hemoglobin (HbA1c), fasting blood glucose concentrations, fasting serum insulin levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and insulin sensitivity.

Results: Our research reveals that when it comes to factors such as several socio-demographic variables, there is no statistically significant difference (p-value ≥ 0.05) between patients who exhibit a positive response to metformin and those who do not. Nevertheless, distinctions were noted in patients' previous history and the duration of their illness, which did influence their treatment response.

Conclusions: Glycemic parameters in individuals with type 2 diabetes can be impacted by a range of factors, such as age, gender, and occupation also it's important to note that these outcomes influenced by additional variables like the adherence for medication, and the existence of diabetes-related complications.

KEY WORDS: genetic polymorphisms, metformin, multidrug and toxin extrusion pharmacogenomics, type 2 diabetes mellitus

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INTRODUCTION

Metformin is the preferred initial treatment for type 2 diabetes because of its strong glucose-lowering capabilities, proven safety, and affordability. Although metformin has multiple effects on glucose metabolism, it is widely agreed that its primary glucose-lowering action in type 2 diabetes patients is mainly through the suppression of hepatic gluconeogenesis [1]. Its primary mechanism of action involves enhancing insulin sensitivity by increasing the expression of insulin receptors and stimulating tyrosine kinase activity [2]. Numerous research studies and clinical trials have provided evidence supporting the effectiveness of metformin either as a monotherapy or in combination with other medications for managing type 2 diabetes (T2D) [3]. Metformin treatment reduces blood levels in type 2 diabetes by affecting multiple pathways, including the reduction of hepatic gluconeogenesis [4]. Approximately 50% of the orally administered metformin dose is absorbed into the bloodstream, after which it is distributed to various tissues [5]. Metformin is present in its unbound form and is eliminated unchanged through renal clearance. Notably, the effectiveness of metformin varies significantly, with over 30% of patients treated with metformin not responding effectively [6]. Recent independent studies have highlighted significant variability in individual responses to metformin therapy [7]. Additionally, metformin treatment has been linked to a higher incidence of gastrointestinal symptoms, ranging from 2% to 63% in different clinical trials, which is notably more common than with most other oral antidiabetic medications. In about 4% of cases, these gastrointestinal symptoms can be severe enough to lead to the discontinuation of metformin therapy prematurely [8].

AIM

This study aims to evaluate how various factors affect various aspects of glycemic control parameters in Iraqi individuals with type 2 diabetes who are with metformin treatment as a single therapy.

MATERIALS AND METHODS

STUDY DESIGN

A cross-sectional study was conducted between April 2022 and June 2023, involving a sample of 150 individuals diagnosed with type 2 diabetes mellitus depending on the American Diabetes Association criteria 2012. These criteria define type 2 diabetes using parameters such as HbA1c levels ≥6.5%, fasting plasma glucose (FPG) levels ≥126 mg/dl, 2-hour plasma glucose levels ≥200 mg/dl during an oral glucose tolerance test (OGTT), or random plasma glucose levels ≥200 mg/dl. The study participants were recruited randomly from the diabetes center at Al-Sadar Teaching Hospital in Najaf, Iraq, and the study received ethical clearance from the Medical Ethics Committee of the Faculty of Medicine at Kufa University.

STUDY POPULATION

The study population comprised 150 individuals with type 2 diabetes, including both males and females, who had been undergoing a monotherapy regimen of metformin tablets (1000 mg) once daily for at least three months duration [9]. These participants range between 30 to 70 years and exclusion criteria for the study encompassed patients with significant organ dysfunction, including heart, liver, and renal failure, individuals above 70 years of age, those with a BMI exceeding 30 kg/m^2, pregnant women, patients with chronic gastrointestinal disorders or malabsorption syndrome, and individuals concurrently using other oral hypoglycemic agents (OHAs) or insulin.

DATA COLLECTION

The process of collecting data involved the investigator using a standardized questionnaire to gather a range of demographic and clinical information from the patients. This information covered details such as their names, ages, body weight, height, duration of the disease, medical history, family history, dietary patterns, sleep routines, and occupations. To calculate the body mass index (BMI), measurements of the weight of the patient and height were taken. Height measurements were taken with patients standing upright, barefoot, arms resting at their sides, and feet together. Weight measurements were recorded with patients standing on a scale, wearing lightweight clothing, and without shoes or socks. The BMI was computed using the formula BMI = weight (in kilograms) / height (in square meters). Patients were then categorized as either normal (BMI<25 kg/m^2), overweight (BMI between 25 to 29 kg/m^2), or obese (BMI ≥30 kg/m^2) [10]. The glycemic control parameters measured include:

• FBG (fasting blood glucose): measured by "RanDox kit-UK", which is rely on the "PAP enzymatic" measurement of glucose.

• HbA1c: the percentage assessed by using immuno assay method by Stanbio/USA kit.

• Serum insulin: assayed according to the procedure recommended by (BTLAB®) company.

• Homeostasis-Model Assessment for Insulin Resistance (HOMA-IR): The approach presented by used the "Homeostasis-Model Assessment for Insulin Resistance (HOMA-IR)" index to measure insulin resistance [11], the of HOMA-IR was calculated in the following manner:

HOMA-IR = Fasting-insulin (μU/L) * Fasting-glucose (mmol/L)/22.5

• Insulin sensitivity: the quantitative insulin sensitivity check index (QUICKI) is derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose [12]:

1 / (log(fasting insulin μU/mL) + log(fasting glucose mg/dL)

BLOOD SAMPLE COLLECTION

For the collection of blood samples, each patient fasted for 8-12 hours overnight, and venous blood was drawn while they were seated. 5 ml of blood was divided into an EDTA tube, with the remaining 2 ml slowly transferred into disposable serum tubes containing a separating gel. Within the EDTA tubes, the blood was allocated for HbA1c assessment using the Immunoassay method. The blood within the serum tubes was left to clot at room temperature for 10-15 minutes, after which it was centrifuged at 3000 × g for approximately 3 minutes. The resulting sera were stored at -80°C until analysis was carried out using the BT LAB® ELISA kit to measure serum insulin, following the recommended procedure provided by the company. Fasting blood glucose levels were determined using the RanDox® kit, which operates based on the PAP enzymatic glucose determination method.

STATISTICAL ANALYSIS

All data was managed by using SPSS version 22, ANOVA test and t-test used for multiple comparisons, and chi-square test for utilization of non-numerical variables. Values of ≤ 0.05 will be considered to be statistically significant.

RESULT

Table 1 shows the sociodemographic data of the participants of the study. 150 T2DM patients with an

Table 1. Socio-demographic data of the participants

Variables		HbA1c <6.5%	HbA1c >6.5%	χ^2	P value
Patients History	No	17	71	30.5713	3.224e-8
	Yes	22	39		
Age		52.00±1.648	53.90±1.82	1.131	0.260
BMI		27.93±0.58	28.01±0.63	0.076	0.940
Duration		6.13±0.94	7.94±0.55	1.681	0.095
Age category	<40	4	6	1.53978	0.4631
	40-50	13	30		
	>50	23	73		
Duration category	<5	22	37	6.5388	0.03803
	5-10	8	43		
	>10	10	29		
Education	Primary	26	82	1.66878	0.4341
	Intermediate	12	22		
	University	2	5		
Job	Yes	12	39	0.388916	0.5329
	No	28	71		
Drugs	No drugs	19	61	0.842973	0.3585
	Other drugs	21	48		
Gender	Male	10	44	2.99042	0.08376
	Female	30	65		
Another related disease	Yes	29	73	0.414034	0.5199
	No	11	36		
HbA1c		26.17%	73.82%	-	-

Table 2. Glycemic variables levels (mean ± SE) in study groups

Variables	HbA1c <6.5%	HbA1c >6.5%	P value
FBC	138.7±9.755	248.62±8.843	0.000
HbA1c	6.036±0.070	9.77±0.203	0.000
Insulin	7.148±1.701	9.57±1.26	0.355
HOMA- IR	45.59±11.55	105.05±14.39	0.019
Insulin sensitivity	1.18±0.08	1.03±0.047	0.587

average age of 53.97±1.86 years and a mean BMI of 28.34±1.58 kg/m^2 (Table 2) were included in the study. Socio-demographic factors such as BMI, age, education, occupation, gender, presence of other medical conditions, past medical history, and dietary habits revealed differences between the two groups. However, these differences did not reach statistical significance (with p-values ≥ 0.05). On the other hand, there was a notable and statistically significant difference between the two groups in terms of disease duration and the patients' medical histories. The patients were classified into 2 subgroups according to their glycemic control; poorly controlled diabetics (HbA1c ≥ 6.5%) who were 73.82% compared to good glycemic control (HbA1c ≤ 6.5%) who were 26.17% (Table 1).

The difference in glycemic parameters between the good and poor responders is represented in table 2. The analysis reveals a substantial disparity in mean glycemic parameters between individuals who responded well and those who responded poorly to metformin treatment. Notably, there were highly significant differences in FBS, HbA1c, and HOMA-IR between these two groups, as indicated by p-values of 0.000, 0.000, and 0.019, respectively. While serum insulin level was observed to be higher in poor responders compared with good responders, but not statistically significant.

Table 3 represents data indicating variations in glycemic parameters (FBS, HbA1c, serum insulin, insulin sensitivity, and HOMA-IR) among good and poor responders about the duration of the disease.

Table 3. Means differences in study variables (mean ± SE) according to duration of disease

Variable	HbA1c <6.5%			P value	HbA1c >6.5%			P value
	<5 years	5 - 9 years	>10 years		<5 years	5 - 9 years	>10 years	
FBG (mg/dl)	122.36±7.21	141.12±16.9	172.70±31.75	0.099	249.32±9.53	246.13±24.95	249.58±18.47	0.843
HbA1C	5.99±0.094	5.98±0.16	6.18±0.14	0.488	9.65±9.21	10.53±0.52	9.34±0.34	0.401
IN	4.81±0.94a	5.56±7.36b	6.34±1.53ab	0.043	10.74±1.6	10.27±1.79	11.58±20.05	0.353
IR	27.98±6.05a	33.21±52.06b	39.86±9.14ab	0.046	118.95±18.76	106.05±17.01	144.67±245.89	0.162
IS	0.94±0.11a	1.41±0.15ab	0.02±0.14b	0.067	1.17±0.06	1.14±0.080	1.09±0.58	0.764

Table 4. Means differences in study variables (mean ± SE) according to gender

Variable	HbA1c <6.5%		P value	HbA1c >6.5%		P value
	Male	Female		Male	Female	
FBG (mg/dl)	124.27±8.61	144.17±13.46	0.378	252.75±15.21	255.76±10.39	0.695
HbA1C	6.16±0.08	5.99±0.09	0.312	9.86±23.40	9.69±0.92	0.088
IN	3.85±0.95	8.44±2.30	0.230	7.65±1.143	10.90±1.98	0.209
IR	24.29±8.34	52.10±15.92	0.294	80.04±10.60	122.25±23.06	0.150
IS	1.90±0.14	1.13±0.09	0.217	1.14±0.055	1.12±0.07	0.794

Table 5. Means differences of study variables (mean ± SE) according to education

Variable	HbA1c <6.5%			P value	HbA1c >6.5%			P value
	Primary	Secondary	University		Primary	Secondary	University	
FBG	138.23±13.49	128.33±11.961	207.00±16.0	0.253	249.28±9.53	256.13±24.958	205.60±30.596	0.543
HbA1C	6.11±0.087	6.03±0.150	6.01±0.200	0.799	9.65±0.21	10.53±0.529	8.52±1.092	0.089
IN	8.76±2.49	4.16±0.849	8.03±4.09	0.469	10.74±1.63	6.24±1.206	3.94±1.071	0.238
IR	72.99±17.25	25.23±5.987	71.63±31.93	0.496	118.69±18.54	68.68±12.615	37.22±10.532	0.230
IS	1.09±0.107	0.94±0.13	1.27±0.24	0.503	1.05±0.060	1.13±0.053	1.95±0.126	0.720

Table 6. Mean differences in study variables (mean ± SE) according to patients' history

Variable	HbA1c <6.5%		P value	HbA1c >6.5%		P value
	No	Yes		No	Yes	
FBG	143.17±15.00	144.22±13.66	0.731	254.95±11.06	260.75±13.91	0.800
HbA1C	6.03±0.119	6.15±0.092	0.081	10.05±0.298	42.23±21.52	0.988
IN	4.67±0.91563	9.06±2.89	0.631	9.40±1.387	9.79±2.28	0.306
IR	31.78±6.806	53.90±20.176	0.711	104.03±15.739	106.17±25.88	0.369
IS	0.97±0.108	0.13±0.120	0.788	1.17±0.055	1.08±0.079	0.195

These differences do not reach statistical significance. Notably, both groups exhibit elevated mean values for glycemic control parameters as the duration of the disease progresses.

Table 4 presents the findings, indicating variations in glycemic parameters (FBS, HbA1c, serum insulin, insulin sensitivity, and HOMA-IR) between good and poor responders' patients. These differences do not reach statistical significance. Interestingly, when comparing genders, females exhibit higher mean values across glycemic control parameters in comparison to males except HbA1c is higher in males.

As documented in table 5, the data reveals differences in glycemic parameters (FBS, HbA1c, serum insulin, insulin sensitivity, and HOMA-IR) between good and poor responders. These differences do not achieve statistical significance. In general, individuals with higher education levels tend to exhibit lower values across most glycemic parameters compared to those with primary and secondary education.

Table 6 illustrates findings that indicate distinctions in glycemic parameters, FBS, HbA1c, serum insulin, insulin sensitivity, and HOMA-IR, between good and poor responders. However, it's important to note that

Table 7. Means differences in study variables (mean ± SE) according to job

Variable	HbA1c <6.5%		P value	HbA1c >6.5%		P value
	Yes	No		Yes	No	
FBG (mg/dl)	126.41±11.597	143.96±13.50	0.426	252.66±15.63	256.40±10.47	0.733
HbA1C	5.91±0.143	6.10±0.082	0.245	15.05±23.08	18.52±8.03	0.415
IN	4.47±1.01	8.33±2.39	0.302	6.31±0.912	11.37±1.872	0.056
IR	27.56±7.950	51.68±16.54	0.351	70.04±10.10	124.18±21.313	0.072
IS	1.96±0.139	1.11±0.102	0.404	1.09±0.05	1.06±0.06	0.513

Table 8. Means differences in study variables (mean ± SE) according to age categories

Variable	HbA1c <6.5%			P value	HbA1c >6.5%			P value
	<40 years	40-50 years	>50 years		<40 years	40-50 years	>50 years	
FBG	106.25±5.80	133.92±14.63	147.04±14.64	0.460	276.33±31.54	238.16±16.10	250.69±11.17	0.622
HbA1C	5.52±0.18a	5.99±0.10ac	5.15±0.093c	0.027	11.00±1.28	9.28±0.34	9.87±0.24	0.152
IN	5.52±2.95	10.66±4.80	5.09±0.93	0.398	15.08±6.15	9.49±2.19	9.08±1.61	0.567
IR	23.27±11.38	70.55±33.60	23.85±5.96	0.320	102.02±68.39	97.00±20.31	102.79±19.27	0.527
IS	1.14±0.24	1.11±0.174	1.01±0.099	0.954	1.40±0.176	1.10±0.10	1.12±0.05	0.377

these differences do not reach statistical significance. Interestingly, all glycemic parameters show higher values in patients with a previous family history of type 2 DM in both groups.

Based on the findings presented in table 7, the statistical analysis indicates that there is no notable difference in glycemic parameters, including FBS, HbA1c, serum insulin, insulin sensitivity, and HOMA-IR, when comparing good and poor responder patients. It is noteworthy that across both good and poor responder patient groups, all glycemic parameters exhibit lower values among individuals who are employed as opposed to those who are unemployed.

Table 8 reveals variations in glycemic parameters FBS, HbA1c, serum insulin, insulin sensitivity, and HOMA-IR between good and poor responder patients. These differences were statistically non-significant. Notably, among good responder patients, there is a significant distinction in HbA1c levels observed across various age subgroups. It's worth mentioning that for most of the glycemic parameters, the mean values are lower in patients aged over 50 years compared to their younger counterparts.

In this study, the correlation between the studied glycemic control parameters in two groups is clarified in table 9. There is a significant strong positive correlation between serum insulin and HOMA-IR, with R = 0.980 and P < 0.000. In addition, HOMA-IR has a significant positive correlation with insulin sensitivity (R= 0.729 and P < 0.000). This means that as insulin sensitivity decreases, insulin resistance increases since they are inversely related. There is a significant moderate positive correlation between HBA1C and FBG, with R = 0.393 and P < 0.000. This means that higher levels of HBA1C are associated with higher levels of FBG. There is a weak inverse correlation between age and HBA1C, with R = -0.072, P = 0.458. There is a significant positive correlation between the duration of diabetes and age, with R = 0.221, and P = 0.020.

DISCUSSION

It is crucial to customize glycemic management for individuals with type 2 diabetes, considering factors such as age, comorbidities, and the risk of hypoglycemia. Effective blood glucose control, with a target HbA1c level below 6.5%, has been shown to substantially reduce the risk of nephropathy and cardiovascular complications [11]. Metformin is actively transported and distributed in the body, and its effects can vary significantly among individuals [13, 14]. We found that the treatment outcome was not affected by the body measurements (such as BMI) of the patient or most of their social and demographic characteristics, as the difference was not statistically significant (p-value ≥ 0.05). However, we did notice that the medical history of a patient and how long they had the disease influenced their response to the treatment. Our finding agrees with a study from Iran that included 103 female diabetes patients who were divided into two groups based on their HbA1c levels. The study from Iran showed that there was no big difference in the social and body measurement data between the two groups using a single-variable analysis. However, using a multiple-variable analysis, the study found that waist size was an important factor for high HbA1c levels [15]. In a study conducted

Table 9. The correlation coefficients among study variables in study groups

		FBG	HBA1C	IN	HOMA-IR	INS	Age	Duration
FBG	R		0.372	0.054	0.136	-0.100	0.383	0.171
	P		0.018	0.740	0.401	0.450	0.015	0.292
HBA1C	R	0.393		0.066	0.149	-0.085	0.390	0.160
	P	0.000		0.689	0.366	0.607	0.014	0.330
IN	R	-0.016	-0.153		0.980**	0.693	-0.188	0.107
	P	0.867	0.112		0.000	0.000	0.253	0.518
HOMA-IR	R	0.159	-0.081	0.959		0.636	-0.175	0.110
	P	0.098	0.401	0.000		0.000	0.288	0.503
INS	R	-0.008	-0.066-	0.775	0.729		-0.129	0.186
	P	0.938	0.495	0.000	0.000		0.435	0.256
Age	R	-0.050	-0.072	-0.014	-0.023	-0.007		0.452
	P	0.602	0.458	0.883	0.808	0.943		0.004
Duration	R	0.007	-0.098	0.087	0.123	-0.085	0.221	
	P	0.945	0.309	0.364	0.202	0.375	0.020	

by Ghazanfari Z et al. [16] was revealed that a longer duration of diabetes was closely linked to suboptimal glycemic control. Our research findings, as shown in table 3, corroborate this observation. We noticed that among both good and poor responder patients, glycemic control parameters tended to worsen as the duration of the disease increased but were statistically non-significant. This finding is in line with numerous other studies that have consistently shown a significant relationship between the duration of diabetes and the deterioration of glycemic control [14]. This phenomenon can be explained by the gradual decrease in insulin production over time, which is associated with dysfunction in beta cells. This might clarify why dietary changes or oral diabetes medications become less successful as the duration of the disease increases [17]. A 2016 study showed that there is a discrepancy in the effect of gender on HbA1c levels. The study reported that males had higher HbA1c levels than females (0.165%, $p<0.0001$). However, this difference was not important clinically except for people aged 30 to 59 years old [18]. Our results agree with this trend, showing that males had a higher mean HbA1c level than females in both groups, as shown in table 4. On the other hand, a study with children who had type 1 diabetes mellitus found a significant rise in HbA1c levels in females compared to males at diagnosis. This difference may be related to the start and timing of puberty [19]. As shown in table 5, our research found that people with higher education had lower average levels of different blood sugar indicators than those with lower education in both groups. This finding agrees with another study that proposes that at least 12 years of education is an essential factor for patients, as it helps them understand their disease better, communicate effectively with healthcare providers, and manage their condition successfully [20]. We investigated the possible link between a patient's diabetes mellitus family history and their blood glycemic control indicators in our recent study. Our results showed that patients who had a family history of diabetes mellitus had slightly higher blood sugar control indicators than those who did not. However, these differences were not statistically significant, as shown in table 6. This finding agrees with another study that also found that people with a diabetes family history had higher levels of blood sugar control indicators, especially HbA1c and fasting plasma glucose levels [21]. On the other hand, some other studies, such as the one by Ghazanfari et al. in 2010, did not find any significant relationship between family history and blood glucose control indicators [22]. We examined the effect of employment status on blood sugar control indicators in our study, as presented in table 7. We observed that the glycemic control indicators were better in employed patients than in unemployed ones. A systematic review and meta-analysis of the literature from electronic databases was done on the therapeutic effect of exercise on blood sugar levels in people with type 2 diabetes mellitus. The review showed that exercise plays a significant role in improving blood sugar control and life quality, BMI, and waist size. Exercise could be a safe and effective addition to drug treatments in these patients [23]. We found that both groups had worse blood sugar control in younger people than in older people, as shown in table 8. This finding agrees with a study by *Sanal et al.* in 2011, which reported that patients who were 60 years old or older had better blood sugar control. The possible explanation for this difference in blood sugar

control between younger and older people could be that younger patients may not take their treatment plan as seriously as older patients [24].

CONCLUSIONS

The glycemic parameters of type 2 diabetic patients are influenced by various factors, such as age, sex, job, patient education, family history, and duration of disease. Some studies have found that younger patients, male patients, unemployed patients, and patients with a family history of diabetes have worse glycemic control than their counterparts. However, these findings are not consistent across all studies and may depend on other variables, such as the type and intensity of treatment, the level of adherence, and the presence of complications. Patient education is an important factor that can improve glycemic control by enhancing the understanding of the disease and self-management skills. Therefore, it is essential to consider the individual characteristics and needs of each patient and provide tailored interventions to optimize their glycemic outcomes.

LIMITATION OF STUDY

One of the main limitations of this study is that cannot establish a causal relationship between the variables of our study. For example, we find that age, sex, job, patient education, family history, and duration of disease are associated with glycemic parameters in type 2 diabetic patients with metformin, you cannot conclude that these factors cause or affect the glycemic outcomes. There may be other factors that influence both the exposure and the outcome, such as lifestyle, or genetic factors. These factors are called confounding variables and they can bias the results of a study.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Najah Rayish Hadi
University of Kufa
299G+HPX, Kufa, Iraq
e-mail: drnajahhadi@yahoo.com

ORCID AND CONTRIBUTIONSHIP

Alaa Abd AL-Hussain Naem: 0009-0005-6658-1121 B C
Mona N Al-Terehi: 0000-0002-9244-6709 B C
Fadhaa Abdulameer Ghafil: 0000-0002-1998-9459 C D
Sahar Majeed: 0000-0002-7296-4998 D E
Najah Rayish Hadi: 0000-0002-8415-5311 A F
Defaf Al-Mudafer: 0009-0008-8200-0800 D E

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

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ORIGINAL ARTICLE

CONTENTS

The state of systemic immunity in congenital cleft lip and palate patients with diseases of oral cavity tissues

Tetiana O. Timokhina[1,2], Oleh I. Holubchenko[2], Olena V. Cherkasova[1], Bohdan M. Melnyk[1]

[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

[2]NATIONAL SPECIALIZED CHILDREN'S HOSPITAL "OKHMATDYT", KYIV, UKRAINE

ABSTRACT

Aim: To determine the state of dental health and the state of systemic immunity in patients in congenital cleft lip and palate patients.

Materials and Methods: The dental status and immunologic tests of 74 patients age 8-18 years old with congenital cleft lip and palate was analyzed: 43 children with unilateral and 31 children with bilateral complete combined cleft lip, alveolar process, hard and soft palate.

Results: Indicators of the prevalence and intensity of the caries process in patients with congenital congenital complete cleft lip, alveolar process, hard and soft palate were high, especially in children with bilateral cleft lip and palate - the decompensated course of caries was determined in 41.93% patients, with unilateral - 23.25%. Chronic catarrhal gingivitis was the most common in both groups of patients - the average severity of gingivitis prevailed - 51.16% with congenital unilateral cleft lip and palate and 51.61% - with bilateral. Atopic cheilitis, glossitis and chronic recurrent aphthous stomatitis were common. This patients have significant changes in the cellular chain of the immune system with a deficiency of the main phenotypes of lymphocytes - CD4+ CD8+ and inflammatory bacterial changes in blood serum.

Conclusions: Patients of unilateral and bilateral complete combined cleft lip, alveolar process, hard and soft palate have significant changes in the dental status and in the cellular chain of the immune system. The level of manifestation of these changes is directly proportional to the extent of localization of the pathology - unilateral or bilateral.

KEY WORDS: caries, congenital defects, cleft lip and palate, diseases of periodontal tissues, diseases of the mucous membrane of the oral cavity, immunological research

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INTRODUCTION

Orofacial clefts include a large group of congenital deformities with structural defects in the oral cavity and adjacent facial structures that occur in isolation, in combination, or as part of other malformations [1]. In Europe, the prevalence of cleft lip and palate is 1.55 cases per 1000 live births [2, 3]. A multifactorial model was proposed to explain the development of orofacial defects [4], about 20% of malformations are caused by genes, 10% by chromosomal mutations, and 70% by multifactorial origin [5, 6]. At the same time, several co-factors can contribute to the formation of congenital cleft lip and palate and aggravate the course of the disease; therefore, a comprehensive multidisciplinary care program is needed to ensure successful treatment outcomes for patients with congenital cleft lip and palate [7].

The leading method of treatment of congenital cleft lip and palate is surgical. But despite the improvement of the technique of surgical interventions, there is some uncertainty about the safety stages of surgical correction of congenital cleft lip and palate (including the suspension of planned operations). Because patients affected by congenital cleft lip and palate, suffer from a high degree of morbidity, in particular, infectious and inflammatory diseases of the oral cavity, respiratory tract and ENT organs (acute respiratory viral infections, acute bronchitis, acute pneumonia) and ENT organs (acute purulent adenoiditis). [8, 9, 10]. Thus, a retrospective analysis of the condition of children with congenital cleft lip and palate (n=339) showed that these children suffered annually more than 10 episodes of uncomplicated and complicated, as well as recurrent, acute respiratory viral infections with the duration of the acute period of the disease up to two weeks, which is a clinical indicator of immunocompromised status. In such cases, the leading diagnosis is secondary immune deficiency, which requires a review of the treatment approach. At the same time, children with bilateral congenital cleft lip and palate, even without cold symptoms, had a significantly higher incidence of perioperative respiratory complications (8.9%) than children with simple cleft lip (1.7%). The authors suggested that clinicians

should consider postponing primary plastic surgery for cleft lip and palate in infants with a suspected presence of a common cold [11].

Immunocompromised patients are prone to a variety of oral manifestations, including oral infections (eg, candidiasis, gingivostomatitis herpetic), aphthous ulcers, and severe periodontal disease, including aggressive periodontitis with premature tooth loss in children/adolescents, and this almost always indicates for the presence of an underlying systemic or local immunological disorder [12-15]. Thus, a clear association was found between severity of inflammation in the oral cavity and defects of local immune protection, which allowed the authors to predict the nature of the clinical course of chronic dental diseases in patients with congenital cleft lip and palate [16-18]. According to modern concepts, the functioning of the immune system and changes in the microbiome of the oral cavity are key

At the same time, despite numerous studies, the features of the functioning of the immune system in children with congenital cleft lip and palate are still being discussed, and in the scientific literature, only isolated data are given that reflect the state of systemic immunity in children with congenital cleft lip and palate. For example, it has been shown that in case of congenital cleft lip and palate, the physiological insufficiency of the immune system and its anatomical compromise lead to a sharp tension of the still immature homeostasis maintenance mechanism in these children [20].

In addition, babies with orofacial clefts are usually born underweight and with impaired immune function from growth retardation [21, 22], and the first stages of surgical treatment of congenital cleft lip and palate relate to physiological age-critical periods of the formation of the immune system [23, 24].

Clinical signs of immune deficiency in children with congenital cleft lip and palate give grounds to suspect defective functioning of the immune system. It is obvious that the solution to this problem and the determination of further therapeutic tactics require a complex pathogenetic approach, which includes an assessment of the features of the functioning of the immune system and, based on the obtained data, the development of new immunotherapeutic approaches aimed at correcting the detected disorders.

Undoubtedly, data on the state of innate and adaptive immune system have an important prognostic value for the successful rehabilitation of patients with congenital cleft lip and palate. Ambiguous, sometimes contradictory data from the scientific literature support the interest in evaluating the features of the functioning of the immune system in young patients with congenital cleft lip and palate.

AIM

To determine the state of dental health and the state of systemic immunity in patients in congenital cleft lip and palate patients.

MATERIALS AND METHODS

The dental status and immunologic tests of 74 children age 8-18 years old with congenital cleft lip and palate was analyzed: 43 children with unilateral complete combined cleft lip, alveolar process, hard and soft palate, and 31 children with bilateral complete combined cleft lip, alveolar process, hard and soft palate. All children were operated on in the Department of Reconstructive and Plastic Microsurgery at National Specialized Children's Hospital "OKHMATDYT" (Kyiv, Ukraine). The patients selected for this study underwent a clinical dental examination - determination of the prevalence and intensity of dental caries, the condition of periodontal tissues and the mucous membrane of the oral cavity. Informed consent in written form was obtained from all the parents for diagnostic studies and treatment methods. The study was conducted in accordance with the principles of bioethics set out in the World Medical Association Declaration of Helsinki - «Ethical Principles for Medical Research Involving Human Subjects» and «General Declaration on Bioethics and Human Rights» (UNESCO). The following subpopulations of T- and B-lymphocytes were determined as diagnosticum erythrocytic for the detection of human T-lymphocyte populations "Anti-CD 3", "Anti-CD 4", "Anti-CD 8", "Anti-CD 16", "Anti-CD 22", Ukraine, Kharkiv city, "Granum" - CD3 + lymphocytes (T cells); CD4 + lymphocytes (T-helpers); CD8 + lymphocytes (T-cytotoxic lymphocytes/suppressors); CD16 + lymphocytes (NK cells), CD22 + lymphocytes (B cells). The phagocytic activity of neutrophils was assessed by the method of determining the bactericidal activity of phagocytic cells (HST test). The study of the functional state of B-lymphocytes was carried out by determining the level of the main classes of serum immunoglobulins Ig G, Ig A, Ig M using immunoturbidimetric method and diagnostic reagent IgA 5+1, IgM 5+1, IgG 5+1 for vitro quantitative determination of IgA, IgM, IgG in blood serum by turbidimetric analysis (Dialab Produktion, Austria). The concentration of circulating immune complexes (CICs) in blood serum was determined by the immunoenzymatic colorimetric method of quantitative determination of CICs C1q in human serum or plasma (NovaTec Immunodiagnostics GmbH).

Data were analyzed with the statistical package IBM SPSS Statistics Base (version 22) and EZR. All results were considered statistically significant at a value of

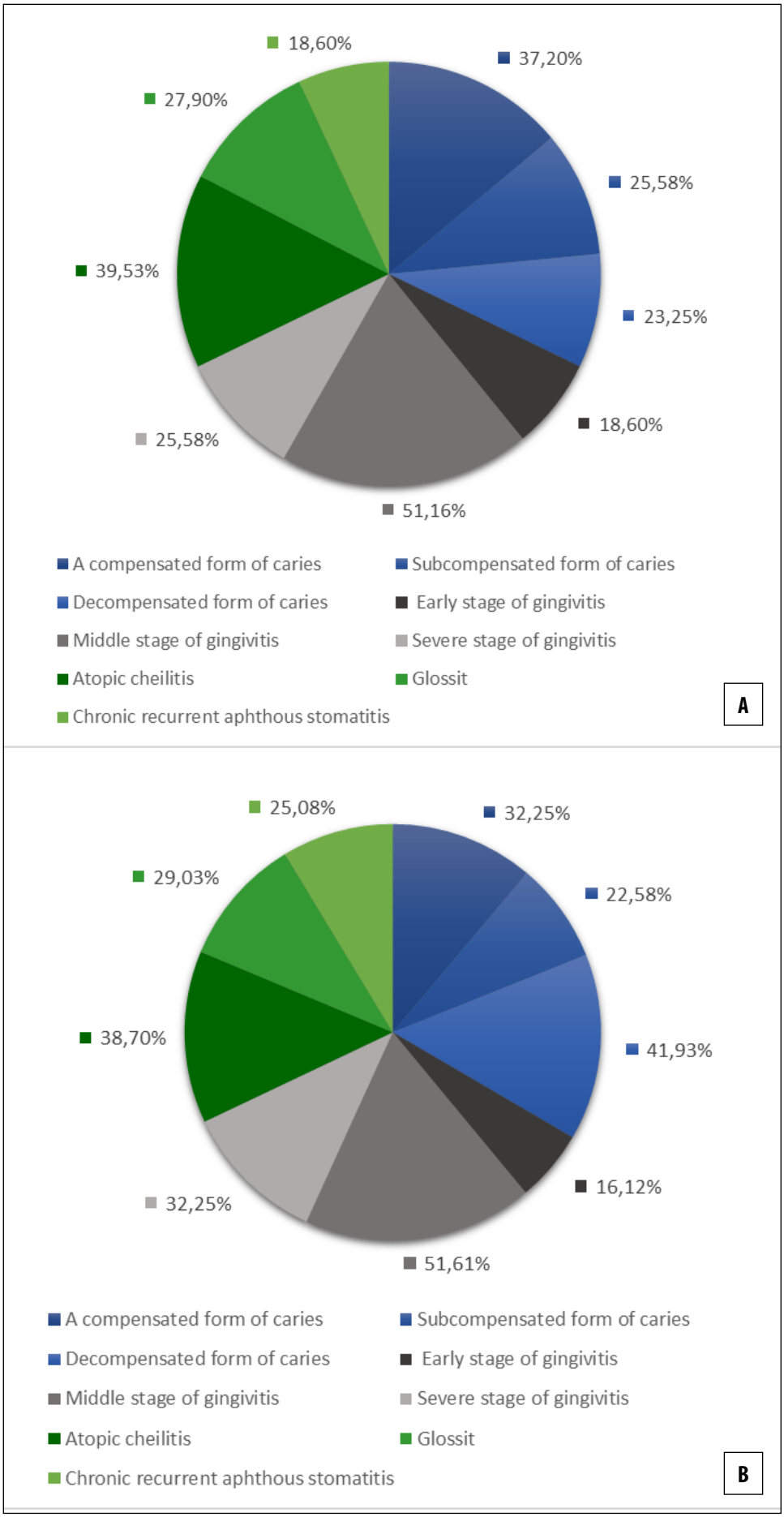

Fig. 1. The prevalence of the oral cavity diseases in children with congenital complete cleft lip, alveolar process, hard and soft palate: unilateral (a) and bilateral (b).

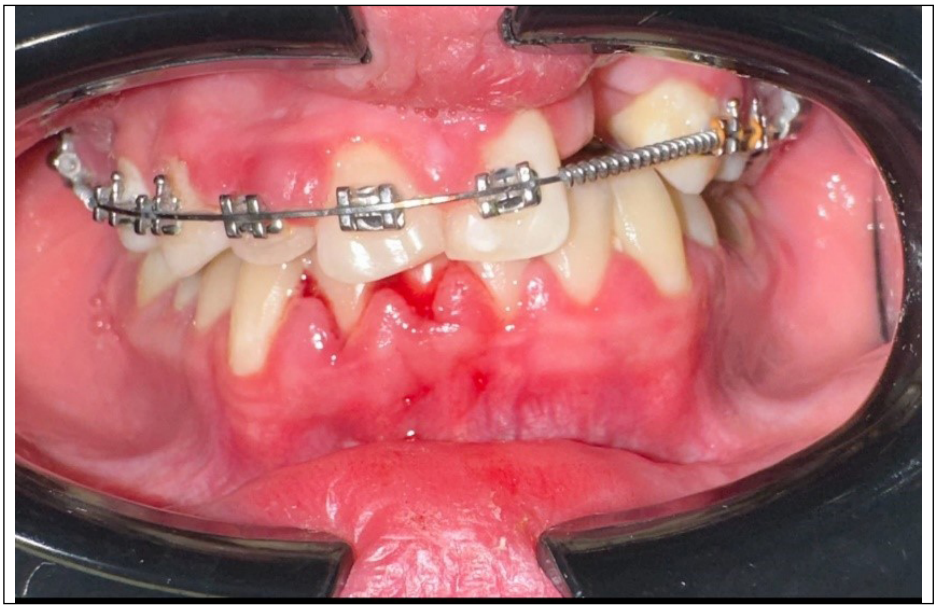

Fig. 2. Patient with congenital unilateral complete cleft lip, alveolar process, hard and soft palate.

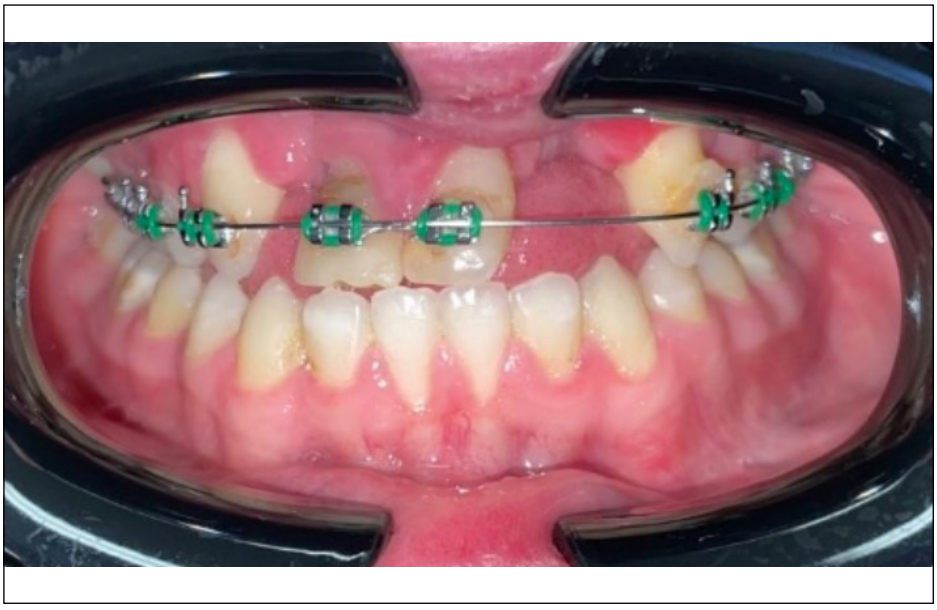

Fig. 3. Patient with congenital bilateral complete cleft lip, alveolar process, hard and soft palate.

$p<0.05$. Quantitative data are presented as mean (M) ± standard deviation (SD), unless otherwise stated. The normality of the data distribution was checked using the Shapiro-Wilk test.

RESULTS

Indicators of the prevalence and intensity of the caries process in patients with congenital congenital complete cleft lip, alveolar process, hard and soft palate were high, especially in children with bilateral cleft lip and palate - the decompensated course of caries was determined in 41.93% patients, with unilateral - 23.25%. Among the inflammatory processes in the periodontal tissues, chronic catarrhal gingivitis was the most common in both groups of patients. The clinical dental examination of the patients determined that the average severity of gingivitis prevailed in the examined patients - 51.16% with congenital unilateral cleft lip and palate and 51.61% - with bilateral. Among the diseases of the mucous membrane of the oral cavity atopic cheilitis, glossitis and chronic recurrent aphthous stomatitis were more common than others (Fig. 1-3).

Patients of both groups underwent an immunological examination, the purpose of which was to identify disorders in the cellular and humoral links of the immune system, as well as possible primary defects of immunity. It is known that primary immunodeficiency diseases can be accompanied by other malformations, including cleft of the hard palate and lip [25]. Among the patients examined by us, there were no patients with signs of primary cellular or humoral immunodeficiency; however, we observed significant changes in the number of the main cellular subpopulations.

The analysis of indicators of the immune system in patients with bilateral combined lesions showed that the total number of leukocytes corresponded to normative indicators. However, it was observed relative lymphocytosis (Table 1).

Indicators of cellular immunity were probably reduced compared to patients with unilateral combined lesions. Thus, the relative number of CD4+ lymphocytes was lower by 21.8% ($p<0.05$), and CD8+ lymphocytes by 29.7% ($p<0.05$). At the same time, a high level of CD22+ lymphocytes was found - by 27% compared to patients in group 2 ($p<0.05$).

A study of the indicators of the immune system in patients with unilateral complete combined lesions showed that in this group of patients the total number of leukocytes was preserved, the relative number of lymphocytes improbably exceeded the indicator of the first group of patients by 12.3%. It should be noted that the content of CD4+lymphocytes, CD8+lymphocytes was probably higher than the similar indicators of the first group, but decreased in comparison with the normative values. Compared to the patients of the first group was found lower relative content of B-cells, which may be caused by relatively smaller inflammatory manifestations in the periodontium and mucous membranes compared to the bilateral combined lesion.

In our opinion, these changes are caused by a long-term inflammatory process in the mucous membrane with long-term persistence of highly pathogenic pathogens. These pathogenic microorganisms destroy periodontal tissues, affect the cellular link of the immune system and also secrete enzymes that cause in the progression of the inflammatory process.

A pronounced deficiency of both CD3+ lymphocytes and the main immunoregulatory subpopulations, namely T-helpers (CD4+ lymphocytes) and T-cytotoxic lymphocytes/suppressors (CD8+ lymphocytes) against the background of a normal total number of leukocytes is a sign of a long-term inflammatory process with decompensation immune response.

It is known that the immune system, like all regulatory systems of the body, necessarily reacts to all pathological processes. In those cases when the activation lasts for a long time or in the conditions of genetic and acquired defects in individual parts of the immune system, the infectious process develops regardless of the antigenic properties of the microorganism, acquires a chronic character, and has a tendency to steady progression [26].

Taking into account the pronounced changes on the part of mucous membranes of an inflammatory nature, the high percentage of detection of isolates of highly virulent pathogens during bacteriological examination was evaluated the concentration of C-reactive protein and procalcitonin in blood serum (Table 2).

In both groups investigating patients found a high level of these indicators that relative to normative values, which indicates an active inflammatory process of bacterial etiology and requires a long-term appointment of antibacterial drugs, taking into account the isolates of pathogens and their sensitivity.

In patients with bilateral combined cleft, the level of both indicators was significantly higher than in patients with unilateral lesions. In addition, also this group of patients has the highest percentage of decompensated forms of caries (41,93%), atopic cheilitis (38,7%) and moderately severe catarrhal gingivitis (51,61%). Combination of mucosal damage with decompensated caries against the background of a large area of damage that creates the conditions for the persistence of highly virulent pathogens, which cause a constant antigenic load on the immune system, and as a result, its "exhaustion" and a relative deficiency of the cellular immune response.

A significantly lower level of C-reactive protein and procalcitonin in the blood serum of patients in 2 group may be due to a smaller percentage of patients with a decompensated form of caries (23,25%) in this group and a relatively high percentage of detection of atopic cheilitis (39,53%) and glossitis (27,9%) in this group of patients. It is the presence of a chronic highly virulent polyresistant bacterial infection that causes a long-term increase in serum C-reactive protein and procalcitonin.

DISCUSSION

Scheuerle et al. in 24 patients with orofacial defects, including: congenital cleft lip and palate studied the existence of an immunological deficiency. Fresh blood samples were analyzed for age-matched immunoglobulin counts, T cells, B cells, and natural killer cells, as well as lymphocyte stimulation and response function.

All patients had certain disorders of the immune system. Seven had specific T-cell abnormalities, and three patients had abnormalities of all test categories. The obtained results indicate that patients with any orofacial defect have abnormalities in the immune system [27]. In 40% of 20 children with severe congenital facial defects had reduced B-cell counts, and 60% had reduced T-cell counts. Chemotaxis was reduced in polymorphonuclear leukocytes in 56% and mononuclear leukocytes in 75% of patients. According to the authors' conclusions, the association of immune abnormalities and congenital facial defects may be more common than previously thought [28].

It is known that after the contact of the immune system with a viral infection, an adequate response from T-lymphocytes is an increase in the number and activity of CD3+ and CD8+ T-cells, which provide specific antiviral immune protection. Inadequate response of CD3+ and

Table 1. The main indicators of the cellular link of immunity in patients with cleft palate (M±m)

Index	1 group	2 group	p
Leukocytes, x109/l	6,51±1,4	6,23±2,31	p>0,05
Lymphocytes, %	41,1±2,06	46,8±1,95	p>0,05
CD3+lymphocytes, %	41,3±1,75	45,7±2,31	p>0,05
CD4+lymphocytes,%	22,6±1,13	28,9±1,02	p<0,05
CD8+lymphocytes,%	20,3±1,16	28,9±1,14	p<0,05
CD4/CD8	1,12±0,11	1,34±0,12	p>0,05
CD22+lymphocytes,%	38,6±1,23	28,9±0,96	p<0,05
CD16+lymphocytes,%	10,9±0,86	13,1±0,83	p>0,05

Notes: p - the probability indicator difference between groups

Table 2. Serum concentration of pro-inflammatory markers in patients with cleft palate (M±m)

Index	1 group	2 group	p
C-reactive protein mg/l	40,1±2,28	20,5±1,68	p<0,05
Procalcitonin, ng/mL	1,05±0,09	0,82±0,06	p<0,05

CD8+ T-cytotoxic lymphocytes to viral antigens indicates defects in the functioning of antiviral immunity, which should be provided by the predominance of adaptive cytotoxic killer cells. The obtained results indicate that natural killer cells play an important role in the control of primary infection by eliminating infected B cells and enhancing the antigen-specific T-cell response by releasing immunomodulatory cytokines [29, 30]. It has been confirmed that in congenital orofacial defects there is a significant decrease in the number of natural killer cells in younger children and their activation in older children. Against the background of immunoregulatory imbalance, there is a decrease in the level of T-lymphocytes at the expense of T-helpers and an increase in the concentration of cytotoxic T-lymphocytes, as well as activation of the B-cell link of immunity. Which indicates the importance of studying the effect of cytokines and their corresponding immunoregulatory cells on the stimulation of the adaptive immune response, which is the most effective protective mechanism for eliminating infectious agents and inflammatory processes [3, 31, 32]. While studying the cytokine status was found that in patients with congenital cleft lip and palate has increased level of pro-inflammatory cytokines, which depends on the clinical form of the cleft. The greatest deviations observed in babies with congenital bilateral congenital cleft lip and palate. The marked changes in cytokine status led to the identification of secondary immunodeficiency in children, which requires immunomodulatory measures [33].

CONCLUSIONS

1. The clinical dental examination of the patients determined that the cariogenic situation is significantly worse in children with congenital complete cleft lip, alveolar process, hard and soft palate - the decompensated course of caries was determined in 41.93% patients. Chronic catarrhal gingivitis was the most common in both groups of patients and the average severity of gingivitis prevailed in congenital bilateral cleft lip and palate - 51.61%. Atopic cheilitis, glossitis and chronic recurrent aphthous stomatitis were more common than others among the diseases of the mucous membrane of the oral cavity.
2. Therefore, patients of unilateral and bilateral complete combined cleft lip, alveolar process, hard and soft palate have significant changes in the cellular chain of the immune system with a deficiency of the main phenotypes of lymphocytes - CD4+ CD8+ and inflammatory bacterial changes in blood serum. The level of manifestation of these changes is directly proportional to the extent of localization of the pathology - unilateral or bilateral.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Tetiana O. Timokhina
Bogomolets National Medical University
1 Zoologichna st., 03057 Kyiv, Ukraine
e-mail: tanyatimokhina@gmail.com

ORCID AND CONTRIBUTIONSHIP
Tetiana O. Timokhina: 0000-0002-0220-0220 A B C D E F
Oleh I. Holubchenko: 0009-0001-2104-6222 A
Olena V. Cherkasova: 0000-0001-7244-0476 D
Bohdan M. Melnyk: 0000-0003-2232-8181 C

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

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ORIGINAL ARTICLE

CONTENTS

Ultrasonographic changes of carotid vessels in patients in the early recovery period of atherothrombotic stroke

Olga J. Mykhaloiko, Tetiana D. Hrytsiuk, Ivanna-Romana M. Fabryka, Oleksandra I. Hotsaniuk, Vira M. Kulaiets, Igor J. Mykhaloiko
IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: To study the state of extracranial carotid vessels in patients with atherothrombotic stroke in the early recovery period (ASERP) according to duplex scanning data.

Materials and Methods: 130 patients in ASERP, were studied. 69 men and 61 women. aged (60.42±7.4) years. Duplex scanning of the vessels of the neck was performed on a Siemens Acuson X 300 device with a linear multi-frequency sensor of 4–10 MHz. The classification of stenozoocclusive lesions of vessels was carried out according to the classification of B.V. Gaidar. Atherosclerotic plaques (AP) are divided into 5 types according to the Nicolaides and Gerulaka classification.

Results: Atherosclerotic stenoses were found in all patients of ASERP: (90%),- in 3.4%. AP type 1 was found in 15% of cases; 2 types - in 33.8%; 3 types - in 26%; type 4 accounted for 12.3% and type 5 accounted for 12.3% of cases. APwhich causing moderate stenosis had a high degree of embologenicity due to the hypoechogenicity and heterogeneity of atherosclerotic plaques of types I, II and III. When the level of stenosis increased, tendency to increase the density and hyperechogenicity of the AP was noted.

Conclusions: 89% patients with ASERP had non-critical, hemodynamically insignificant stenoses of the carotid arteries. Types II and III AP, mostly of an eccentric structure, dominated. Moderate stenoses were more often caused by echo-negative atherosclerotic layers, which is a source of increased embologenicity, and stenoses of a greater degree, for the most part, were echo-positive.

KEY WORDS: stroke, atherosclerotic plaque, degree of stenosis, carotid basin

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INTRODUCTION

Detection of atherosclerotic lesions of the carotid arteries and subsequent slowing or stopping of the progression of atherosclerotic plaques with the help of pharmacological effects significantly reduces the risk of recurrent stroke in patients with a history of atherothrombotic stroke [1]. The most affordable, non-invasive method of examining cerebral arteries to detect changes is ultrasound diagnostics (US). The accuracy of this method is confirmed by the high degree of correlation of the measured indicators with the results of the study of the material after carotid endarterectomy. When examining brachiocephalic arteries, the following parameters are evaluated: patency, geometry, diameter, state of the vascular wall (integrity, surface, thickness of the intima-media complex, degree of differentiation into layers); the state of the lumen of vessels: the presence of atherosclerotic layers, their localization, extent, echogenicity and degree of obliteration. For the clinician, the characteristic of atherosclerotic plaque is of particular interest, along with determining the degree of stenosis [1-4].

Image analysis of atherosclerotic plaque identifies three distinct textural patterns: hypoechoic (corresponding to lipid-rich plaques and hemorrhages), iso- or moderately hyperechoic (fibrous or fibrofatty plaques), and hyperechoic with shadowing (calcified plaques). Hypoechoic or dyshomogeneous plaques with neovascularization, surface irregularities have a greater tendency to ulceration, embolization and are more prone to clinical complications than hyperechoic, calcified, homogeneous plaques with a smooth surface and lack of neovascularization [3-6]. However, despite the fact that plaque characteristics and echomorphology help to identify high-risk patients, in current practice, the degree of stenosis remains the main determinant of clinical decisions. Atherosclerosis is considered an etiological factor of stroke if there is confirmatory dopplerographic or angiographic evidence of extracranial or intracranial artery occlusion or stenosis of more than 50% [2, 7].

It should be noted that statin therapy causes biochemical remodeling of atherosclerotic plaque with

a greater effect on lipid components than on total plaque size [3, 8].

Pathological deformations of extracranial carotid arteries occupy a special place among the causes of ischemic disorders of cerebral circulation. According to observations, ICA abnormalities detected on ultrasound occur in 10-43% of the population of patients without atherosclerotic lesions of the ICA, diabetes and hypertension [2, 5]. At the same time, ICA kinking is observed in 5-25% of patients with symptomatic cerebrovascular insufficiency, or in patients with identified stenosis of the internal carotid artery.

AIM

The aim of the study is to study ultrasonographic changes in the extracranial carotid vessels in patients with atherothrombotic stroke in the early recovery period.

MATERIALS AND METHODS

A clinical and laboratory analysis of 130 patients with an atherothrombotic stroke in the early recovery period (ASERP) (from 3 to 6 months after an acute stroke in 2020-2023) was conducted. In this time range, when patients were referred to the Ivano-Frankivsk Regional Clinical Hospital for the purpose of rehabilitation therapy, an ultrasound duplex scan of the vessels of the neck was repeated, the results of the examination were entered into the patient's outpatient card (f. 025/o). All subjects were taking antiplatelet agents, statins, hypotensive and hypoglycemic drugs for the purpose of secondary prevention of stroke when indicated. Carotid endarterectomy was recommended for patients with hemodynamically significant atherosclerotic stenoses. Among the examined were 69 men and 61 women aged (60.42±7.4) years.

Exclusion criteria were: the presence of severe somatic pathology in the stage of decompensation, oncological diseases, patients with an unknown cause of stroke, patients with cardioembolic and lacunar subtypes of strokes.

Ultrasound duplex scanning of the vessels of the neck was performed on a Siemens Acuson X 300 device with a linear multifrequency sensor from 4–10 mHz) according to standard methods. At the same time, the level of damage to extracranial carotid arteries, the degree of stenosis and the hemodynamic significance of atherosclerotic lesions, the morphological structure of atherosclerotic plaque, its surface, and the pathological tortuosity of extracranial arteries were evaluated.

The classification of stenotic-occlusive lesions of cerebral arteries into hemodynamically significant and insignificant was carried out according to the classification of B. V. Gaidar et al. [3]. According to these criteria, stenosis of the main artery of the 1st degree was diagnosed when the vessel narrowed to 40% of the diameter, 2nd degree – 40–60%, 3rd degree – 60–75%, 4th degree – 75–90%, 5th degree – more than 90%, hemodynamically significant considered stenosis IV–V degree [3, 11].

The nature of the atherosclerotic plaque (AP) according to the classification of Nicolaidese and Geroulaka distinguished five types of atherosclerotic plaques: type I: only echonegative echonegative («soft» homogeneous plaque); II type: mostly echonegative with more than 50% hypoechoic areas (heterogeneous hypoechoic plaque); type III: mostly echo-positive with more than 50% hyperechoic areas (heterogeneous hyperechoic plaque); IV type: only echo-positive («dense» homogeneous plaque); V type: pronounced calcinosis, which gives an acoustic shadow.

Statistical processing of the obtained data was carried out using the statistical analysis program IBM SPSS Statistics 26. The frequency of qualitative indicators is presented in absolute (n) and relative (%) frequencies with 95% CI values in the form «n (%; 95% CI)».

The assessment of the reliability of the differences in the obtained results in different groups of observations and the testing of the null hypothesis was carried out by calculating the Pearson's chi-square ($\chi 2$) correspondence criterion.

RESULTS

Atherosclerotic stenoses were found in all patients of ASERP: (<40%), - in 20% of cases, (40-60%), - in 36.9% of cases, (75-90%), - in 7.6% and (>90%), - in 3.4% (Table 1). The localization of the atherosclerotic plaque, which was more than 50%, corresponded to the side of the affected hemisphere. In 27% of cases, a combined lesion of the vessels on both sides of the carotid vessels was noted. At the same time, the side of the affected hemisphere corresponded to the side of atherosclerotic layering with greater stenosis in 20% of cases. In the remaining 7% where hemodynamically insignificant stenoses were ascertained, the side of the affected hemisphere corresponded to the side of localization of the hypoechoic plaque.

The majority of patients (89%) had non-critical, hemodynamically insignificant stenoses of the carotid arteries up to 75%. In these subjects with a clinical picture of ischemic stroke, heterogeneous atherosclerotic plaques were found in the basin of the middle cerebral arteries and the anterior cerebral artery, and different variants of the plaque coating were noted.

Table 1. Level of atherosclerotic stenosis and morphological structure of atherosclerotic plaque according to duplex scan results

Level of stenosis	Type of atherosclerotic plaque															p
	I n=20			II n=44			III n=34			IV n=16			V n=16			
	abs.	P, %	±m	abs.	P, %	±m	abs.	P, %	±m	abs.	P, %	±m	abs.	P, %	±m	
<40% (n=26)	6	30,0	10,25	10	22,7	6,32	6	17,60	6,54	4	25,0	10,83	-			=0,751
40–60 % (n=48)	8	40,0	10,95	19	43,2	7,47	14	41,20	8,44	4	25,00	10,83	3	18,8	9,76	=0,367
60-75% (n=42)	6	30,0	10,25	14	31,8	7,02	13	38,20	8,33	5	31,30	11,59	4	25,0	10,83	=0,913
75-90% (n=10)	-			1	2,3	2,25	1	2,90	2,90	2	12,50	8,27	6*/#	37,5	12,10	<0,001
>90% (n=4)	-			-			-			1	6,30	6,05	3	18,8	9,76	=0,600

Notes:

* - statistically significant difference compared to the indicators of the group of patients with type II atherosclerotic plaque;

- statistically significant difference compared to the indicators of the group of patients with type III atherosclerotic plaque.

Table 2. Ultrasound characteristics of atherosclerotic plaques in patients in the early recovery period of atherothrombotic stroke

Ultrasonic characteristics of AP	Patients with ASERP (n=130)	% (95 % CI)
Atherosclerotic plaques:		
- eccentric	97	74,6(66,2-81,8)
- circular	33	25,4(18,2-33,8)
AP surface:		
- smooth	32	24,6(17,5-32,9)
- unequal	22	16,9(10,9-24,9)
- with decay	10	7,7(3,8-13,7)
- with an ulcer	14	10,8(6,0-17,4)
- combined	52	40,0(31,5-49,0)

Thus, AP type 1 (homogeneous, hypodense or "soft") was found in 15% of cases; 2 types (heterogeneous with a predominant hypodense or "soft" component) - in 33.8%; 3 types (heterogeneous with a predominant hyperdense or "dense" component) - in 26%; type 4 (homogeneous hyperdense or "dense") accounted for 12.3% and type 5 (with pronounced calcinosis, which produces an acoustic shadow) accounted for 12.3% of cases. A statistically significant difference was found between the specific gravity of people with atherosclerotic plaques of type V and the proportion of people with type II ($p<0.001$) and III ($p<0.001$) atherosclerotic plaques. No significant difference was found between the proportions of individuals with IV and V type of atherosclerotic plaques ($p>0.05$).

Atherosclerotic layers causing moderate stenosis (up to 60%) had a high degree of embologenicity due to the heterogeneity of atherosclerotic plaques of types I, II and III. These results prompted the correction of therapy in the form of an increase in the dose of the statin used. With an increase in the level of stenosis of the internal carotid artery, a tendency to calcification of atherosclerotic plaque was observed.

Most of the atherosclerotic layers, 97 (74,6; 95 % CI 66,2-81,8 %) had an eccentric structure of AP, in 33 (25,4; 95% CI 18,2-33,8 %) AP were circular (Table 2).

In 32 (24.6; 95% CI 17,5-32,9 %) patients, the surface of AP was smooth, in 22 (16,9; 95% CI 10,9-24,9 %) - uneven, in 10 (7,7; 95% CI 3,8-13,7 %) - with disintegration, in 14 (10,8; 95% CI 6,0-17,4 %) - with ulcer, in 52 (40,0; 95% CI 31,5-49,0 %) - with a combined surface.

In 17 (13%) patients, ICA tortuosity was detected, of which 11 (64%) were C-shaped, 6 (36%) were S-shaped. 13 (10%) patients had unilateral tortuosity, 4 (3%) had bilateral tortuosity. Deformations with a local increase in blood flow rate twice or more in the bending zone were considered hemodynamically significant. In most cases, ICA deformations were combined with atherosclerotic vessel damage.

DISCUSSION

The degree of stenosis of the carotid vessels has long been used as the main factor for determining the risk of stroke and is an indication for treatment [1, 3]. However, additional characteristics of the plaque, such as the morphology and surface of the plaque, which are often not taken into account by clinicians, play an equally important role in the occurrence of vascular disasters, which explains the term «vulnerable plaque» [4].

According to the results of our study, despite the use of statins for the secondary prevention of atherothrombotic stroke, we found the presence of potentially dangerous unstable atherosclerotic plaques of types I and II in almost half of the examined patients. At the same time, soft, unstable atherosclerotic plaques were noted mainly in patients with hemodynamically insignificant stenoses up to 60%. It follows from this that the risk of recurrent stroke in such patients is also high, as fragments of atherothrombotic layers, breaking off, can at any time create an obstacle to cerebral blood flow. This fact is explained by the insufficient effect of statin therapy on lipid metabolism and remodeling of atherosclerotic plaques and necessitates correction of the applied therapy.

Our findings are similar to the results of the NASCET study, which found that unstable atherosclerotic plaque increased the risk of neurological symptoms approximately fourfold compared with stable plaque. Researchers Simon A, Gariepy J, Chironi G and others. argue that even an increase in the thickness of the wall of the common carotid artery is considered a reflection of arterial inflammation and is a predictor of cerebrovascular events in the short term, and conversely, dense calcified plaques are less often associated with ischemic events and may be a protective feature of plaques, preventing thrombus aggregation or providing additional mechanical stability plaque surfaces. The same data were reported in a systematic review by Kwee RM. Where symptomatic plaques have been shown to have less calcification than asymptomatic plaques.

However, there are other, completely opposite opinions reflected in the meta-analysis (Brinjikji W, Rabinstein AA, Lanzino G, etc.) who claim that the instability of the atherosclerotic plaque detected by ultrasound does not correlate with neurological symptoms and does not pose a danger for the patient.

Given the fact that statins affect mainly the change in the structure of plaques, and not the degree of stenosis, their use in the early stages is logical and necessary. According to the results of a study of prospective ultrasound monitoring of ulcer plaques during the year (Schminke U, Motsch L, Hilker L) it was established that the vast majority of carotid plaques (76.5%) remained unchanged, 23.5% of ulcers regressed, while only 5.8% ulcers progressed. Consistent with these results, another research group followed carotid plaques for 2 years and concluded that 67% of carotid plaques showed no change during follow-up, 6% regressed, and 5% progressed [8].

That is, the understanding of the fact that atherosclerotic plaque can progress and transform from stable to unstable even on the background of statin therapy confirms the need for dynamic duplex scanning of the vessels of the neck in order to detect potentially embolic plaques.

CONCLUSIONS

During duplex scanning of the carotid vessels, it is important to pay attention not only to the degree of stenosis, but also to the echomorphology of the atherosclerotic plaque, since hypoechoic layers that cause even a slight degree of stenosis often provoke clinical complications.

According to the results of our study, 89% of patients with ASERP had non-critical stenoses of the carotid arteries, up to 75% with a clinical picture of ischemic stroke in the basin of the middle and anterior cerebral arteries. The vast majority of atherosclerotic plaques in the examined persons had an eccentric structure 97 (74.6; 95% CI 66.2-81.8) with a combined surface 52 (40.0; 95% CI 31.5-49.0).

In 74 patients (57%), atherosclerotic layers caused stenosis (< 60%) and had a high degree of embologenicity due to the heterogeneity of atherosclerotic plaques of types I, II and III. When the level of stenosis of the internal carotid artery increased, a tendency to calcification of the atherosclerotic plaque was observed, so hemodynamically significant stenoses (75-90%) were caused mainly by echo-positive («dense» hyperechoic) plaques of IV and V types (<0.001).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olga J. Mykhaloiko
Ivano-Frankivsk National Medical University
2 Halytska st., 760008 Ivano-Frankivsk, Ukraine
e-mail: myhalojko@i.ua

ORCID AND CONTRIBUTIONSHIP

Olga J. Mykhaloiko: 0000-0003-0618-4501 A B C D E F
Tetiana D. Hrytsiuk: 0000-0001-9150-4148 E F
Ivanna-Romana M. Fabryka: 0000-0002-1154-3241 E F
Oleksandra I. Hotsaniuk: 0000-0001-9691-7419 E F
Vira M. Kulaiets: 0000-0002-3224-4291 E F
Igor J. Mykhaloiko: 0000-0002-6288-1806 C E F

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

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ORIGINAL ARTICLE

CONTENTS

Chemo-sensory loss and FUT2 gene in COVID-19 infected Iraqi dentists

Ferial Mahmood Abdulrida, Ameena Ryhan Diajil
ORAL DIAGNOSTIC SCIENCES DEPARTMENT-ORAL MEDICINE, COLLEGE OF DENTISTRY, UNIVERSITY OF BAGHDAD, BAGHDAD, IRAQ

ABSTRACT

Aim: To find any association between specific ABO blood groups and *FUT2* secretory status and COVID-19 in a sample of Iraqi dentists.

Materials and Methods: For each participant, a questionnaire including demography, COVID-19 status, blood grouping, and RH factor, with chemo-sensitive symptoms was recorded. The saliva samples were collected and DNA was extracted from leukocytes. Sequencing of molecular detection of the *FUT2* gene by real-time PCR and the data was done, whilst drawing the phylogenetic tree.

Results: Out of 133, most of the dentists were female 61%, most were just under 35 years of age. The most participants in this study were predominantly with blood group O (40%), followed by B, A, and AB, with (90%) of them were RH+. All blood grouping and RH factor were high significantly associated with COVID-19 infection and its frequency ($p<0.001$). A significant association between smell dysfunction and infected blood group A and RH+ ($p=0.044, 0.038$) while taste dysfunction was negatively and significantly correlated with AB group ($r=-0.73$; $p=0.008$). The *FUT2* secretor showed a significant association with COVID-19 infection and frequency. The majority of COVID-19-infected participants experienced a significant loss of both smell and taste with fast recovery within 2 weeks.

Conclusions: The COVID-19 infection susceptibility and reinfection are associated with *FUT2* secretory status and greatly associated to olfactory and gustatory sense loss.

KEY WORDS: ABO system, COVID-19, non-secretor status

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INTRODUCTION

Susceptibility to COVID-19 is not so different from other infectious diseases [1]. The Oligosaccharide structures, unique to antigens, are formed through the action of glycosyltransferase enzymes, products of primary gene. The sugar molecules attach to the Oligosaccharide chain by enzymes. Antigens of blood groups, considered secondary *gene products, found on cell surface of RBCs, platelets, leukocytes, plasma proteins, specific tissues, and enzymes also are presented in body secretions, like saliva, gastric secretions, amniotic fluid, breast milk, seminal fluid, sweat and urine [2]. The ABO blood type system includes antigens A, B, and H. Individuals with blood type A could face a higher susceptibility to the infection than those with blood type O [3]. Pathogens may express antigens resembling the type A blood group. Types O and B produce antibodies against type A antigens, reducing their susceptibility. In contrast, types A or AB, lacking anti-A antibodies, might exhibit higher susceptibility to the pathogen due to the absence of this specific immune response [4]. Anti-A antibodies demonstrated the capability to hinder the interaction between spike protein and ACE2 [5]. The half-life of Von Willebrand factor and factor VIII is longer and higher concentrated in individuals with blood group A unlike other blood groups [6]. The enzyme called glucoside 2-alpha-L-fucosyltransferase 2 production are coded by Fucosyltransferase 2 (FUT2) gene. Which determines the ABO antigens secretor status [7]. The size of FUT2 gene 9,980 bases with a plus-strand orientation, is located on chromosome 19q13.33 and it comprises of two exons, with lengths of 118 and 2,995 base pairs, respectively, an intron of 6,865 base pairs separates them. The first exon serves as a non-translated coding region, while the 343 protein amino acids are coded in second exon [8-10]. Fucosyltransferase 2 'SE' is an Autosomal dominant pattern, secretor gene, 'whereas 'se' is the recessive form [11]. A population lacking ABH antigens in secretions is characterized by the presence of a non-functional (mutated) gene, non-functional secretor gene (FUT2) a non-secretor status, had only (FUT1) a single functional H gene, resulting in the expression

of H antigen only on the (RBCs) but absent in their secretions [12]. International Society of Blood Transfusion (ISBT) represented 29 null and weaker alleles identified for the FUT2 gene (ISBT 018). The se^{428} (Trp143stop) allele is a highly prevalent non-secretor allele among Caucasians. It is employed in genome study research that the (rs601338), or (G428A) was occurred due to mutations in the second exon of the FUT2 gene, the single nucleotide polymorphism (SNP) rs601338. It is a stop codon at 143 a.a position (Trp-Ter) more common in Iranians, and Africans beside Europeans [13]. Genetic diversity within FUT2 is estimated to be as ancient as three million years. At least 1.87 million years ago, the 428G>A mutation occurred [14]. Researchers have observed that this polymorphism plays a regulatory role in the innate immune response and to evaluate the continuation of the human race when facing pathogen outbreaks [15]. Additionally, it has indirect effects by modifying the microbiota or serving as a nutrient source for pathogens [16]. The epidemiological cause for linking ABO blood types, in addition to multiple clinical, developmental, and genetic factors all can be affected epithelial tissues' inheritance the secretor status and pathogens exposures [17] and carcinoma and ulcers. Non-secretors, particularly those with blood type A, are linked to lower levels of infections, caused by yeast (Candida) and Streptococcus or gastric bacterial overgrowth. Moreover, individuals who are non-secretors have a heightened susceptibility to Crohn's disease among other autoimmune disorders. Those patients with significant symptoms of respiratory illness and influenza A virus, rhinovirus, or respiratory syncytial virus were Secretors overrepresented patients [16]. A high expression of the secretor (H) antigen in postnatally alive newborns saliva and tracheal aspirates, was found [17]. The susceptibility associated with the FUT2 gene may have implications for vaccine development and study design (1). The study aims to find any association between specific ABO blood groups and *FUT2* secretor status and COVID-19 in a sample of Iraqi dentists.

AIM

Aim of this work was to find any association between specific ABO blood groups and *FUT2* secretory status and COVID-19 in a sample of Iraqi dentists.

MATERIALS AND METHODS

SAMPLE COLLECTION

According to STROBE guidelines for cross-sectional studies [18], this study was conducted on Iraqi dentists from Baghdad City working in primary health centers and private clinics. Sample collection was done from 9 March-21 September 2022 after acquiring the approval of the Ethics Committee for Research from the College of Dentistry, University of Baghdad, Baghdad, under protocol number 460722. A consent form was signed by each volunteer's participant before starting. A questionnaire for each participant was filled out by one examiner after being validated by experts, including demographic information, medical history, COVID-19 infection status, and vaccination date and type. The dentists were asked about the onset, duration, the ABO blood grouping, and eventual regression of the chemo-sensitive symptoms.

Exclusion criteria for these study participants including the exclusion criteria for CCCRC test for olfactory assessments:

- Prior surgical procedure or radiation therapy in mouth or nose.
- Already present manifestation of altered gustation or smell.
- Previous history of trauma in head.
- Allergic rhinitis, chronic rhinosinusitis.
- Psychiatric or neurological disorders or treatments.
- Dentists vaccinated by other than Pfizer or Astra-Zeneca.
- Dentists refused to continue this study and disagreed to give blood and saliva samples or not in contact with patients.

SALIVA COLLECTION

According to Garbieri et al. (2017), the saliva was collected and stored for DNA extraction (19), supplement A.

MOLECULAR DETECTION OF ALPHA (1,2) FUCOSYLTRANSFERASE (FUT2) GENE

DNA extraction from saliva: saliva in a polyethylene tube was used for DNA extraction procedure by Using Favor prep: genomic DNA mini kit; the kit is special for blood/cultured cells; (100 preps) for research use only cat.no: FABGK 100 lot no: CB 827122103 from Favorgen biotech corp. Taiwan (WWW.FAVORGEN.COM). The extracted DNA was determined by using Agarose gel Electrophoresis.

PRIMER DETECTION

The primer was detected and analyzed by the National Center for Biotechnology Information (blast.ncbi.nlm.nih.gov/blast.cgi) USA and primer 3 pulse bio-informatics (bio-informatics.nl/cgi-bin/primer3plus/primer3plus.cgi)

Table 1. Blood groups in total sample according to variables

Infected blood groups count	Frequency of infection			Association (Fisher's Exact)	Correlation Spearman
	one time	twice	> 2 times	P Value	P Value
O	19	11	7	<0.001	r=-0.81;<0.001
A	8	2	3	<0.001	r=-0.91;<0.001
B	14	6	2	<0.001	r=-0.90;<0.001
AB	3	4		0.001	r=-0.89;<0.001
TOTAL	44	23	12	<0.001	r=-0.88;<0.001
RH	40	21	10	<0.001	r=-0.88;<0.001
	Olfactory clinical dysfunction				
	Ansonia	hyposomnia		P Value	
		severe	Moderate	Mild	
O	3	19	11	5	0.47
A	1	4	6	2	0.044
B	3	7	8	4	0.16
AB		3	2	2	0.6
TOTAL	7	33	27	15	0.09
RH+	7	31	23	11	0.038
	Gustatory dysfunction				
	Agusia	Hypogeusia		P Value	
		Severe	Moderate	Mild	
O	0	7	11	15	0.21
A		3	0	8	0.27
B		3	6	9	0.53
AB			1	3	0.1
Total		13	18	35	0.013
RH+	0	11	17	31	0.07
Total RH	0	13	18	35	0.012
	loss of both sense				
	Both	Taste only	smell only		
O	17	2	4	0.001	r=0.3;0.03
A	6	3	2	0.003	0.71
B	14	0	2	0.001	r=0.33;0.036
AB	4	1	1	0.1	0.86
TOTAL	41	6	9	<0.001	r=0.21;0.015
RH+	35	6	8	<0.001	r=0.22;0.014
	Lasting to recovery/days				
	1-15	>15-30	>30		
O	15	2	6	0.008	r=-0.46;0.001
A	5	4	2	0.003	r=-0.63;0.002
B	9	5	2	0.002	0.29
AB	1	2	3	0.09	r=-0.67; 0.014
Total	30	13	13	<0.001	r=-0.4;<0.001
RH+	26	12	11	<0.001	r=-0.4; <0.001

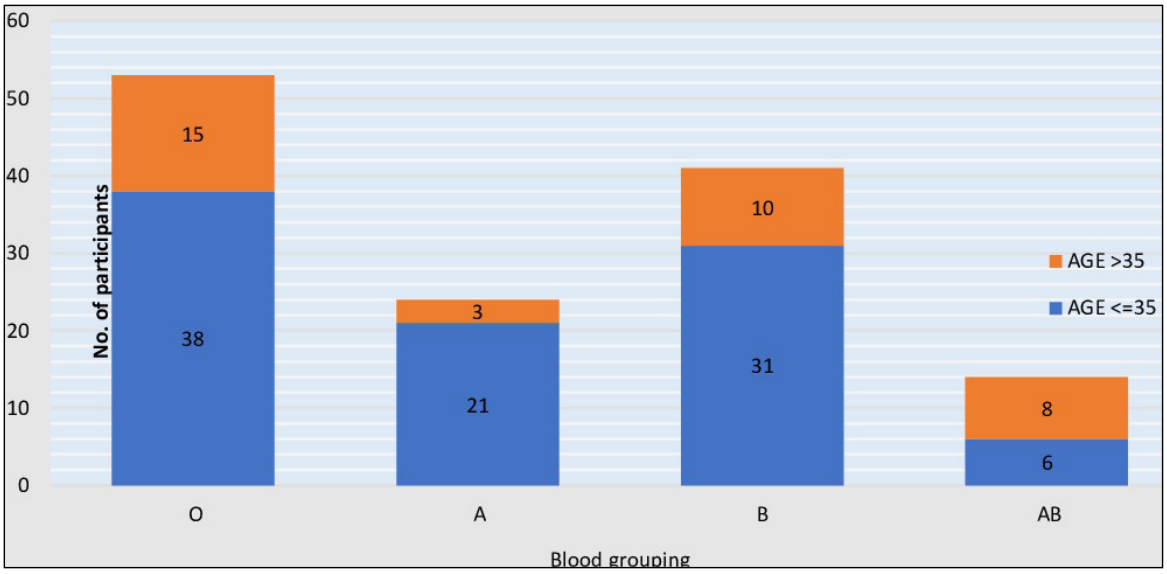

Fig. 1. Age and blood groups of the total sample.

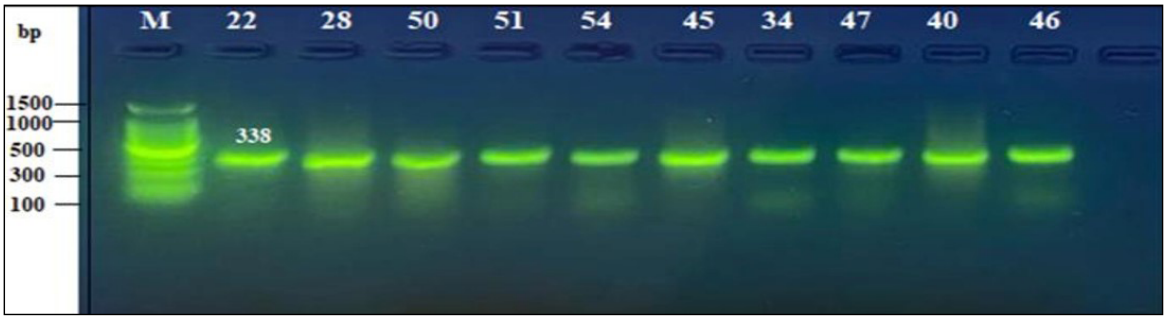

Fig. 2. The gel electrophoresis profile results of 338 bp of FUT2 partial gene band using 2 % Agarose stained with a red safe DNA dye and electrophoresed by 5vol/cm in TBE buffer lane M.DNA Marker 100 bp.

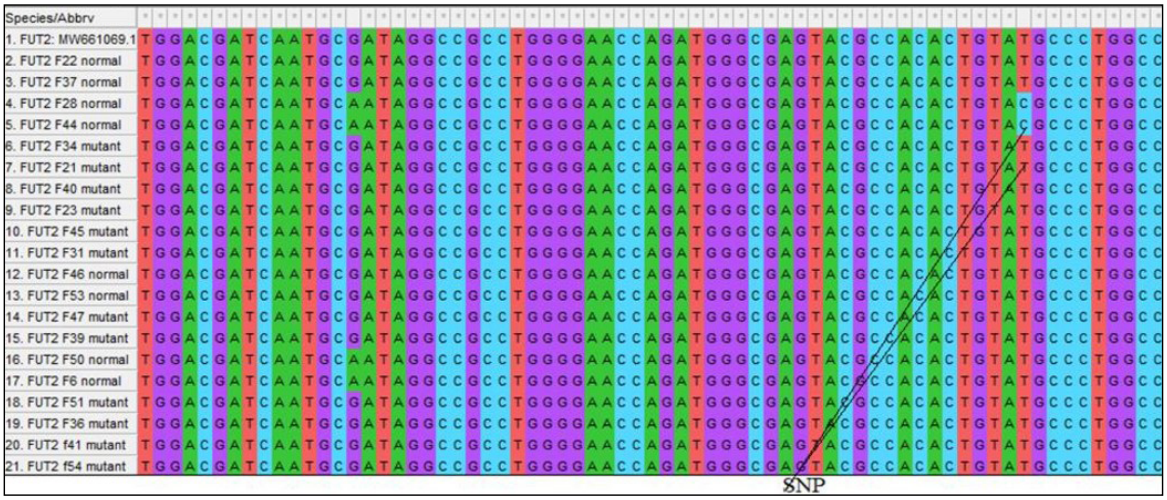

Fig. 3. The substitution (Transition) at 171A>G; and at 216C>T both non sense mutations.

DETECTION OF FUT2 GENE BY PCR

The procedure started by using a kit for the previous synthesis primers for FUT2 (rs601338) Foreword primer (F); Internal oligonucleotide (I); and Reverse primer (R). The kit's name Alpha AND, S.E.N.C. date 17/8/2022. (Synthesis number 696328; name of oligonucleotide F428; sequence (5´ to 3´) od -269 nm).

SEQUENCING

The PCR products of the FUT2 gene for each sample were amplified using both primers forward, reverse. Subsequently, sequencing was done after sending amplified products to Korean company, 'Macro Gene'. Data

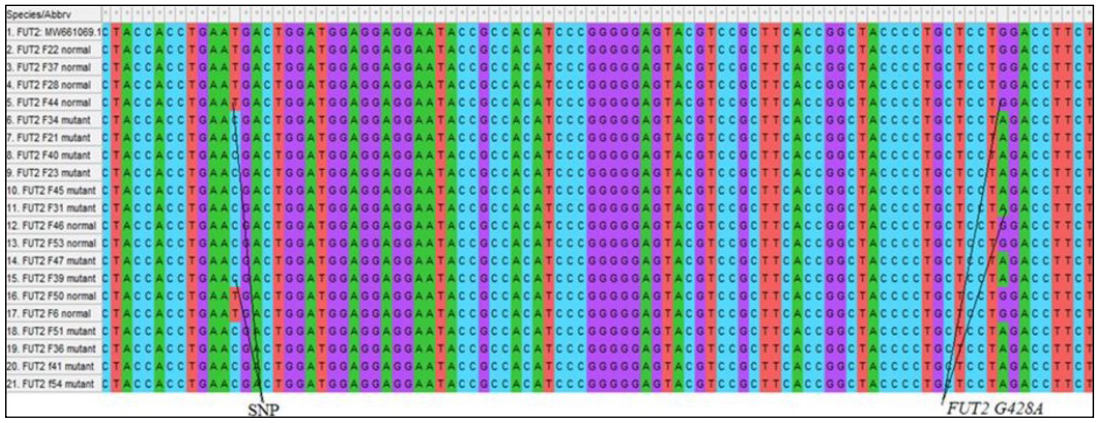

Fig. 4. Nucleotide sequence of sense flanking the partial FUT2 gene compared with the related identity to gene bank strains: MW661069.1 of FUT2 gene using the MEGAX program with non-sense transition at 357T>C.

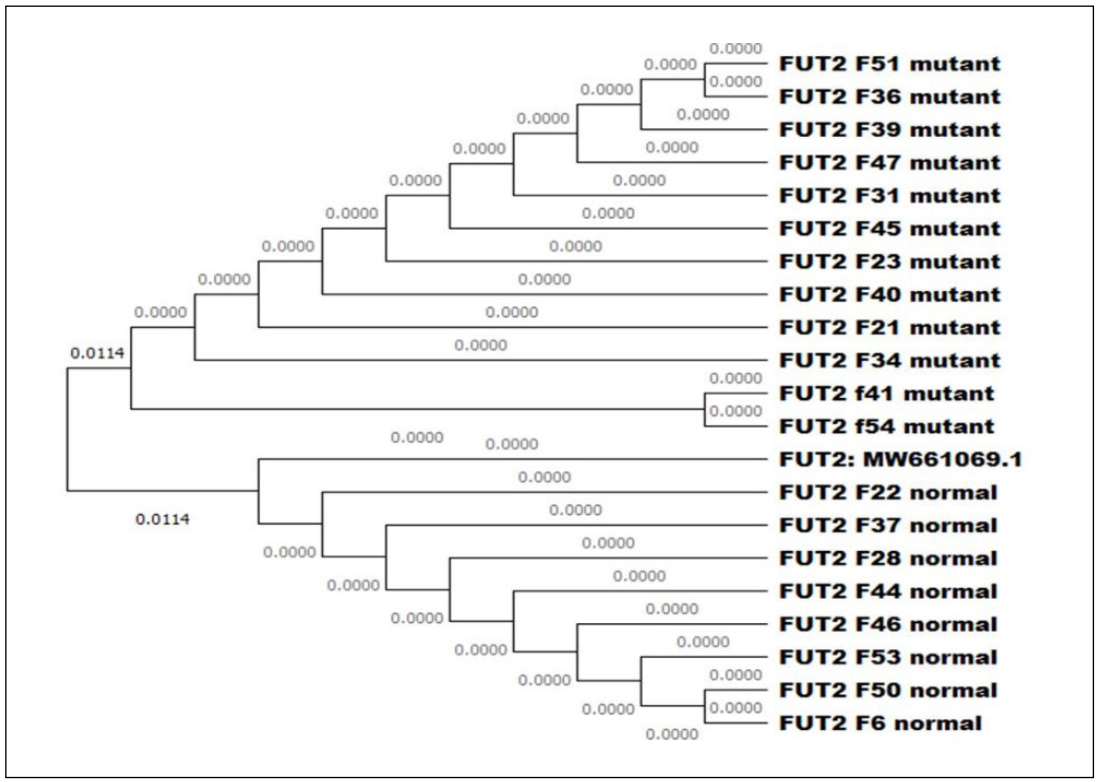

Fig. 5. The Maximum Likelihood method was used to infer the evolutionary tree of the flanking region of FUT2 G428A mutant. In the analysis, 21 nucleotide sequences were examined, comprising a total of 47 positions in the final dataset. MEGA X was used to perform the evolutionary analyses.

that sequenced underwent analysis using sequence analyzer software, specifically Finch software. Nucleotides were then subjected to BLAST (Basic Local Alignment Search Tool) analysis using specialized software to identify and compare sequence similarities (http://www.ncbi.nim.nih.gov). Using the 'MEGA 6.0 version' software, through 'UPGMA method' was employed to construct the phylogenetic tree.

STATISTICAL TESTS

Data was expressed using mean/standard deviation and frequency/percent according to the type of the variable. Normally distributed data the independent sample t test was used and for non-normally distributed data the Mann-Whitney U test was used to assess the difference between 2 continuous variables. Kruskal-Walli's test and ANOVA test were used alternatively to assess the difference between more than 2 independent continuous variables. Chi-square test and Fisher's exact test were used alternatively to assess the association between categorical variables. Correlation tests were used to assess the strength and direction of association between the studied variables. A significance level of 95% with a P-value was deemed significant in this analysis if equal to or less than 0.05.

Table 2. Nucleotide substitution in FUT2 partial gene located on chromosome 19 p13.3 isolated in Iraq.

No. of sample	Type of substitution	Location	Nucleotide	Range of Nucleotide	Sequence ID	Expect	Identification
12	Transition	272	G>A	1-285	OR453929	2.00E-138	99%
8	Transition	272	G>G	1-285	OR453928	4.00E-145	100%

Table 3. FUT2 gene association to COVID-19 infection status

Variable	Secretor	FUT2 GEN		P-Value
		Non- Secretor		
Infection	PCR +	7	5	0.053
	PCR -		7	
	NO PCR	1		
Clinical olfactory	Anosmia	1		r=0.2, p=0.048
	severe	3	4	
	moderate	2	6	
	mild	2	1	
Frequency of infection	Once	1	3	0.008*[1]
	Twice	5		
	>twice	1	2	
	None	1	6	
Loss of sense	Both	6	1	0.21
	Taste		1	
	Smell		1	
	none	2	9	
Recovery time	1-15 days	2	2	0.16
	>15-30 days	2		
	>30days	2		
	none	2	9	
Duration from last vaccine	0-6 Mon.	3	3	r=0.22, p=0.037
	>6-12 Mon.	4	5	
	>12Mon.	2	3	
	none			

*Significant value. [1] Fisher's Exact test. r Spearman correlation.

RESULTS

DEMOGRAPHICAL AND CLINICAL FINDINGS

Out of 133 Iraqi dentists were included in this study, (52; 39%) male and (81; 61%) female. The age range was 24-67 (years) and the age mean was 31.36 (±9.09 years). The samples were divided into two age groups equal and below 35 years old (97; 73%) and above 35 years old (36; 27%). The clinical features included systemic disease, participants suffered from systemic diseases were limited (24; 18%) and these systemic diseases were diabetes milieus, hypothyroidism, and anemia. A positive infection was found in 60% of our participants, 34% once, 17% twice, and 9% more than twice.

BLOOD GROUPING AND RH FACTOR

The predominant blood group among 133 participants was O+(46, 34.5%) followed by B+ (36, 27%), A+ (24, 18%), AB+ (14, 10.5%), O- shown in (8, 6%), B- (4, 3%) and AB- was only1 (1%) Using Fisher's Exact, a significant association was detected between blood groups and age (p =0.034).While no significant association was found between RH factor and both age and sex in the study groups, this could be shown in (Figure 1) as high infected blood grouping individuals were young age. All blood groups were highly associated and correlated negatively and significantly with both positive COVID-19 infection and its frequency of recurrent infection, beside of that infected group A and RH+ were associated significantly with olfactory

dysfunction (p=0.044; 0.038) respectively, whereas taste dysfunction was shown in both infected total blood and RH groups (0.013; 0.012) respectively. High negative significant correlation was reported between infected AB blood group and taste dysfunction (r=-0.73; 0.008). Both senses smell and taste loss were associated with infected blood groups O, A, B, RH+ (0.001, 0.008, 0.001, <0.001) respectively, while a positive significant weak correlation was detected between infected blood groups O, B, RH+ (r=0.3; 0.03, r=0.33; 0.036, r=0.22; 0.014) respectively. The recovery time was occurred faster within first 2weeks and significantly in infected group O, A, B, RH+ (0.008, 0.003, 0.002, 0.001) respectively whereas the negative significant correlation was found in infected groups O, A, AB, RH+ (Table 1, Fig. 1).

THE MOLECULAR DIAGNOSIS

Genomic DNA was extracted from all study sample saliva and gave perfect concentration (20-100 ng/µl) and purity (1.7-1.9), the extracted DNA was analyzed by gel electrophoresis and visualized under UV light as a clear band.

THE PCR REACTION FOR *FUT2* SECRETOR GENE

The amplification of the *FUT2* gene flanking region fragment with 338 bp showed a clear sharp band in the total sample when electrophoresis on 2% agarose gel, Fig. 3, Fig.2.

THE SEQUENCING

Sequencing was done for 20 samples selected randomly from whole sample groups. The mutation in allele G428A (at 272 of the sample sequences) appeared in 12 samples out of 20 selected samples and the associated nonsenses at 357 T→C (201 of sample sequence) the substitution occurred with no changes in the amine acid expressed Asparagine AAT(AAU)→AAC as AAT--AAU(RNA) as both AAU, AAC nitrogen bases expressed the same amine acid Asparagine so the mutation not sense; another substitution occurred on location 216 C→T (60 of sample sequence) the substitution occurred with no changes in the amine acid Tyrosine as both TAC (UAC)→TAT (UAU) both expressed the same a.a. And last one at 171 as A→G in nitrogen bases related to amine acid Alanine GCA→GCG as both GCA, GCG both related to same amine acid so no sense mutation associated, but both substitution mutations associated with a main mutation on G428A non-secreter gen samples than normal secreter samples, Fig. 3., Fig.4.

A PHYLOGENIC TREE

A phylogenic tree was drawn for the main mutation in this study, 12 mutants with and 8 normal with the bank gene standard sequence, (Fig. 5).

GENE BANK SUBMITTING AND ID

The non-secretor and secretor sequencing of this study selected samples was sends to gen bank and ID was sent from NCBI gen bank for two sequences, Table 2.

THE *FUT2* GENE ACCORDING TO OTHER VARIABLES

The normal secreter *FUT2* gene appeared in infected person more than non-infected one, although no significant association with infection was detected. There was a significant association between the secreter gene and frequency of infection with COVID-19 (Fisher Exact test=14.7, p =0.008), the normal secreter participant had more times of infection than non-secreter One, Table 3.

A low positive significant correlation exhibited between this gene and olfactory dysfunction (spearman correlation=0.2, p=0.048), and same correlation with last vaccine duration with secretor status (r=0.22, p=0.037), Table 3.

DISCUSSION

The distribution order of blood grouping ABO was (O>B>A>AB) and (RH+>RH-); the COVID-19 rate among patients in this study decreased in the order of (RH+, O, B, A, AB). Partially approximate with order showed by Ray (O> A>B>AB) [20]; different countries studies exhibited the same order of Ray, Saudi Arabia, United Arab Emirates (UAE), and USA [21]. Both COVID-19 infection and frequency were significantly associated with blood grouping and RH+ mostly in RH+ and blood group O than in other blood groups; no direct link between each specific blood group and COVID-19 infection was found; agreeing with Anderson [22]. Unlike a previous Iraqi study that associated blood group A and men's sex to COVID-19 susceptibility [23]; no such association was found, a lower sample size of this study if compared to previous could be the cause. Despite varying rates of ABO admitted persons, in different populations, blood groups A and O tend to be more

prevalent in those studies [23], while O and B, in contrast, were higher in this study population. More hazards and susceptibility was noted in group A, and more protection and asymptomatic in group O in previous studies [21]. Study group O, participants despite their high percentage of COVID-19 infection and frequency along with RH+, showed mild signs and symptoms/or no symptoms at all; which agreed with the Turkish study [24]. The oligosaccharides blood antigens act as receptors for coronaviruses, N-acetyl galactosamine is an extra sugar in group A. It is absent in group O making an expression of the virus more in group A [25]. Anti-A antibodies bind to COVID-19 S protein limiting its binding to ACE2 in epithelial cells, [26] decreasing initial infection, and delaying the spread of the virus, which is influenced by the titer of ABO iso-agglutinins and the incidence of blood group O [27]. Greater olfactory loss occurred in groups A and RH+. Besides, it is more prominent in group A blood group if compared to group O, when both had sense loss with frequent infection of COVID-19. The blood concentrations of ABO glycoproteins changing are affected by several factors like inflammation, endothelial function, and microvascular coagulation. The environmental factors and racial divergence may alter associations of certain blood groups with certain infections [28], and could explain, besides ABH gene polymorphisms, an increase in ACE1 levels associated with increases in the predisposition to cardiovascular problems and severe cases of COVID-19. Regarding the RH+ group, individuals exhibited a greater chance for a positive result for SARS-CoV-2, while individuals with Rh-negative (Rh–) blood group may experience a protective effect against severe illness caused by SARS-CoV-2. This could be explained by most of the population with RH+ grouping [29]. Taste dysfunction varied from mild to severe scoring, which showed in infected and recurrent RH+ and group O different levels of ACE between blood groups affected the lower risk or severe symptoms [30] and high expression of ACE2 in keratinized mucosa and taste bud [31]. The anosmia and ageusia were unnoticed among COVID-19 patients, unless an objective test was done, and it was not interpreted as a severe COVID-19 manifestation [32]. This agreed with this study. A lower level of ACE which converted angiotensin I to II, which is a recruited inflammation reaction, increasing blood pressure, makes group O with low risk of severe symptoms. The rs601338 (W154X) is identified as the most prevalent inactivating variant [33]. The rs601338 variant results in the expression of the FUT2 enzyme with significantly low activity, as a significant association was recorded between the COVID-19 infection and frequency with the secretor status than non-secretor ones agreed with the increased proportion of secretor antigens and infectious diseases especially virus. Histo-blood group antigens (HBGA), such as the H antigen, may play a role in mediating the attachment of pathogens, leading to infections [34]. Recognizing of cell attachment spike protein to A-type HBGA causes a higher vulnerability of secretors compared to non-secretors [35], while the non-secretor or carriers of FUT2 non-functional mutations are protected from infection [36]. Secreter participants showed more sense loss and a higher recovery rate than non-secreter ones. This is due to a modification of the innate immune by molecular mimicry. The complex glycan of (S) protein structure can support ABH epitopes [37]; and are extensively expressed in various tissues, respiratory and gastric mucosa, and endothelium, kidney, and heart tissues [38]. Prolonged diarrhea, vomiting, severe disease, and greater infection transmissibility were shown in secreters than non-secretors [39]. The proportion of FUT2 was considered during the study of the efficacy of the vaccine, so, Pfizer type of vaccine was significantly related to secreter status in participants who received 2 doses of Pfizer. The gap among studies could be accredited to sample sizes, ABO heterogeneity, genetic differences in viral strain, phenotypes variation in blood groups across countries, as well as genetic differences in *FUT2* mutation, which might influence the assortment of COVID-19 clinical phenotypes [40].

CONCLUSIONS

The frequent blood group varies according to the population that they take. The A-antigen and H antigen of ABO, HBGA groups are acting as receptors for coronavirus; this increased susceptibility and severity of infection and more dysfunction associated. On the other hand, the anti-A antibodies, RH- and non-secreter act as protectors from the infection.

ETHICAL APPROVAL

The Ethics Committee for Research from the College of Dentistry, University of Baghdad, Baghdad, under protocol number 460722

LIMITATIONS

The small sample size taken and the lack of control groups made the comparison difficult.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Ferial Mahmood Abdulrida
University of Baghdad
University intersection, Baghdad Governorate, Baghdad, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP

Ferial Mahmood Abdulrida: 0000-0002-2141-1314 B C D E
Ameena Ryhan Diajil: 0000-0003-3387-1104 A E F

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

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ORIGINAL ARTICLE

CONTENTS

Vitamin D receptor gene rs2228570 (FOKI) polymorphism associated with essential hypertension in Iraqi patients

Aseel R. Jabir[1], Hussein A Saheb[2], Bassim I Mohammad[1], Ahmed M Sultan[2], Sinaa Abdul Amir Kadhim[1], Asma A Swadi[1]
[1]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, COLLEGE OF MEDICINE, UNIVERSITY OF AL-QADISIYAH, IRAQ
[2]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, COLLEGE OF PHARMACY, UNIVERSITY OF AL-QADISIYAH, IRAQ

ABSTRACT

Aim: To understand how vitamin D receptor gene polymorphism (VDR rs2228570) affects blood pressure in Iraqi patients with essential hypertension in Al Diwaniya province.

Materials and Methods: This is a single-center observational cross-sectional descriptive study of 90 patients with essential hypertension. Using the PCR-TETRA ARM technique, blood samples were genotyped and examined for the polymorphisms of FOKI (rs2228570) gene.

Results: The most frequent allele was A (121, 67%) while the most frequent genotype was AG (55, 61%). There was no statistical difference between the actual and expected frequency distribution, according to Hardy-Weinberg equilibrium. The effect of VDR polymorphism rs 2228570 on blood pressure indicates (the mean systolic blood pressure in AA, AG, and GG carrier patients was 149, 150 and 166 respectively, P=0.29. On the other hand, the mean diastolic blood pressure in AA, AG, and GG carrier patients was 89, 89, and 94 respectively P=0.6) there was no statistically significant effect on systolic and diastolic blood pressure.

Conclusions: there is no statistically significant effect of VDR rs2228570 on SBP and DBP (p = 0.6), vitamin D receptor gene polymorphism rs2228570 was related to vitamin D level.

KEY WORDS: single nucleotide polymorphism, rs2228570, essential hypertension, vitamin D receptor gene

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INTRODUCTION

Blood pressure is commonly expressed as the ratio of systolic to diastolic pressure (the pressure the blood exerts on the artery walls while the heart contracts and relaxes) [1]. A systolic blood pressure (SBP) of 140 mm Hg or higher, or diastolic blood pressure (DBP) of 90 mm Hg or higher, is considered as hypertension [2]. Primary and idiopathic hypertension is other names for essential hypertension. This type of high blood pressure is the most common in 90 to 95 percent of people. Essential hypertension appears to be largely influenced by genetic factors [3]. Hypertension is the greatest cause of death worldwide. Because the primary symptoms of hypertension are difficult to detect, many cases (40-50%) commonly go untreated, gaining it the term "silent killer" [4]. It is estimated that genes that govern vitamin D levels are causative of 30-50% of the BP fluctuations [5]. Identifying the genes that regulate vitamin D levels in essential hypertension may provide a more accurate factor in understanding the disease's molecular pathophysiology. The RAAS plays an important role in the physiologic control of sodium and potassium balance, intravascular volume, and blood pressure [6]. Excessive RAAS activity is now well documented to raise the cardiovascular risk that is minimized by reducing or blocking the RAAS [7]. Low vitamin D levels have been linked to clinical outcomes previously associated with increased RAAS function, such as hypertension, inflammation, and CVD [8]. Animal investigations showed that 1,25(OH)2D3-VDR complex adversely regulates renin expression and that vitamin D-induced RAAS activity can prevent poor vascular outcomes to the same extent as pharmaceutical angiotensin receptor antagonism [9]. This theory has been supported by human studies, which showed that reduced circulating vitamin D concentrations are associated with higher plasma renin activity and angiotensin II concentrations [10, 11] and that vitamin D deficiency is associated with higher RAAS activity, which can be reduced with vitamin D3 therapy intervention [9, 12]. Fok I (rs2228570) is a VDR gene SNP that has received a lot of attention. This polymorphism was discovered in the early 1990s, which is comprised of T to C mutation in exon-2. Because the mutation occurs in a start codon (ATG), the C variation causes an alternate start site to be employed, resulting in a larger protein [13]. The Fok I polymorphism can result in shortened proteins and is connected with an increased risk of hypertension. The

Table 1. PCR primers with their sequence, amplicon size, and annealing temperature

Primer	Sequence	Amplicon	Annealing
rs2228570 (FOKI)	Inner forward 70 AAGTGCTGGCCGCCATTGCCTACA (24)	A-allele 201 bp. G-allele 257 bp. Two outer primers 404 bp.	69°C
	Inner reverse 70 CCGTGGCCTGCTTGCTGTTCTTACAGGTAC (30)		
	Outer forward 70 AAAATGCAAGGGCTCCCTTCATGGAAACA (29)		
	Outer reverse 70 CGAAGGCACTGTGCTCAGGCCTGG (24)		

Table 2. Substances used in study, their producer, and their place of origin

No.	Chemical	Company and Origin
1	TBE buffer	Intron (Korea)
2	Agarose	MarLiJu (Korea)
3	Ethidium bromide	BioBasic (Canada)
4	Ladder	Bioneer (Korea)
5	Primers	Macrogen (Korea)

Table 3. JNC8 classification of blood pressure [19]

Category	Systolic		Diastolic
Normal	<120	and	<80
Pre-hypertension	120-139	or	80-90
Hypertension (Stage 1)	140-159	or	90-99
Hypertension (Stage 2)	>160	or	≥100

rs2228570 polymorphism is induced by thymine to cytosine transfer, which causes a translational frameshift defined by an expansion of the open reading frame to the next initiation codon (ATG), leading to the synthesis of a shortened 424 amino acid protein. ATG-encoded methionine (M1 form) was found in the f allele, whereas ACG-encoded methionine (M4 form) was present in the F allele [14]. The shortened protein in people with the FF genotype is hypothesized to increase the development of essential hypertension by boosting renin and angiotensin II production [15, 16]. It is suggested that the shortened protein's transcription activity is more than that of the full-length protein. Furthermore, the shortened protein's enhanced reactivity to 1,25(OH)2D3 may change VDR function as well as vitamin D in cells and tissues [17]. This shows that 1.25(OH)2D3 can suppress renin expression in humans and raise the risk of cardiovascular and metabolic disorders [18].

AIM

The aim of this research is to understand how vitamin D receptor gene polymorphism (VDR rs2228570) affects blood pressure in Iraqi patients with essential hypertension in Al Diwaniya province.

MATERIALS AND METHODS

STUDY DESIGN, PATIENTS RECRUITMENT, SETTING AND TIMING

The study involved 90 people (50 men and 40 women). This is a single-center observational cross-sectional descriptive study for hypertension patients of Iraqi nationality who have been diagnosed using the Eighth Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. All candidate patients were evaluated and diagnosed by a cardiologist or other professional caregiver. The study, which lasted from July 2022 to July 2023, was carried out at the Al-Diwaniyah teaching hospital and the Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiya, Iraq. The laboratory work was done in the Al-Qadisiyah University's Department of Pharmacology and Therapeutics in the province of Diwaniyah.

QUESTIONNAIRE FORMULA

The following data were collected from the patients: name, age, sex, race, senior status, patient identification number, comorbidities, diagnosis, serum lipid level, uric acid, glucose, blood urea, and serum creatinine.

ETHICAL CONSIDERATIONS

The College of Medicine at Al-Qadisiyah University's Ethics Committee approved the study, and all patients received an explanation of the procedures before providing their informed permission.

PRIMERS USED IN THE CURRENT STUDY

Using the PCR-TETRA ARM technique, blood samples were genotyped and examined for polymorphisms in the FOKI (rs2228570). The Integrated DNA Technologies (IDT) website and data from the National Center for Biotechnology Information (NCBI) were used to create polymerase chain reaction (PCR) primers for the VDR

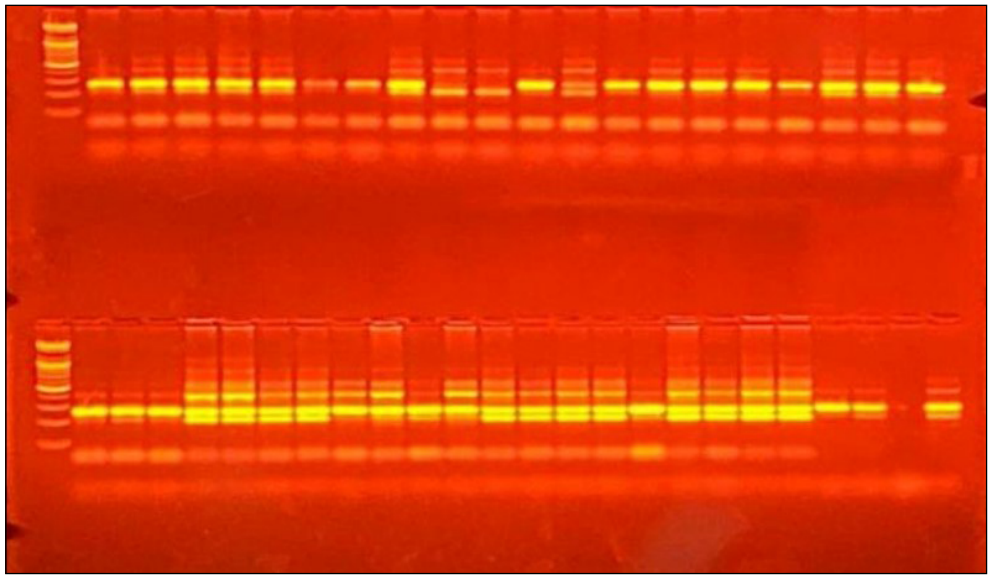

Fig. 1. Image of electrophoresis of agarose gel that demonstrates the analysis of PCR product of FOKI (rs2228570) gene from certain blood samples of patients.

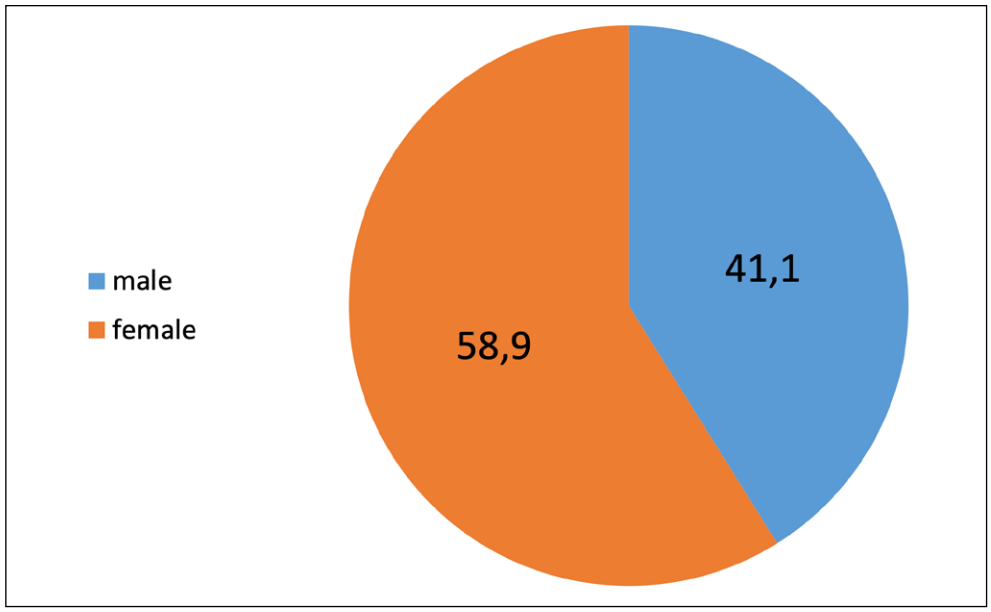

Fig. 2. Sex distribution of participant hypertensive patients in the study (n=90).

FOKI (rs2228570) genes. The primers were designed especially for this study using information for SNP sequence available online (https://www.ncbi.nlm.nih.gov/), SNP data and online tool for tetra arms primer design (http://primer1.soton.ac.uk/primer1.html), and for sequence SNP primer design (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). The complementary nature of the given primers was further verified using the BLAST gene bank tool to ensure that they were not complementing the non-targeted gene. Deionized distilled water (DDH2O) was used to dissolve the lyophilized primers in the master tube to obtain a concentration of 100 pmol/l. Then, 10 pmol/µl was created as the working solution by transferring 10 µl from the master tube to another tube and bringing the total volume to 100 µl with DDH2O (Table 1).These primers were provided by Bioneer Company, Korea.

CHEMICALS THAT WERE USED IN THE CURRENT STUDY

Table 2 shows the chemical compounds employed in this investigation, along with their place of origin and Production Company.

BLOOD SAMPLING

4ml of blood was taken from each patient, divided into two parts, and aspirated from the antecubital vein.

- 1ml of the patient's whole blood was taken in an EDTA-containing tube for DNA extraction and kept at -20 C until DNA extraction.
- 3ml of the patient's whole blood was collected in a gel tube, spun for five minutes at 5,000 revolutions per minute, and the serum was collected for use in biochemical assays.

Table 4. Effect of VDR polymorphism rs 2228570 on blood pressure

Genotype rs2228570	Systolic BP means	SE	P value	Diastolic BP mean	SE	P value
AA	149	2.7		89	1.2	
AG	150	1.9	0.29 NS	89	0.9	0.6 NS
GG	166	1.5		94	6	

Table 5. Effect of VDR polymorphism rs 2228570 on blood pressure

Genotype rs2228570	Numbers	Mean Vid D level	S.E	P value
AA	33	4.4	0.44	
AG	55	7.5	1.05	0.028
GG	2	9.8	4.3	

BLOOD PRESSURE MEASUREMENT

A mercury sphygmomanometer was used to take blood pressure (BP) measurements. Before getting the measurements, the patient was instructed to sit comfortably and rest for five minutes with her right arm unclad and her legs uncrossed. The palm of the right arm was then raised and put on the table. The proper cuff size was selected. The cuff was kept at the same level as the heart during measurements. Patients are classified in the Eighth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC8) as shown in table 3.

GENOTYPING

Genomic DNA from blood samples was extracted by using a DNA isolation kit (Frozen Blood) Geneaid, USA.

PCR–TETRA ARM TECHNIQUE

Using the PCR-TETRA ARM technique, blood samples were genotyped and examined for polymorphisms in the FOKI (rs2228570) (Fig.1).

STATISTICAL PROCESSING

SPSS version 25 was used to make the statistical analysis. The data were shown as mean ± SE. Allelic and genotypic frequencies for each SNP were calculated. P value <0.05 was considered statistically significant.

RESULTS

DEMOGRAPHIC DATA

This study included 90 Iraqi hypertensive patients, 58.9% (n=53) were females and 41.1% (n=37) were males (Fig.2), with the mean ± SD age (years) of the study group 53.2±13.8, and mean ± SD BMI of 29±5 kg/m^2.

EFFECT OF VDR POLYMORPHISM RS 2228570 ON BLOOD PRESSURE

As shown in Table 4, the mean systolic blood pressure in homozygous AA, heterozygous AG, and homozygous GG carrier patients was 149, 150, and 166 respectively. On the other hand, mean diastolic blood pressure in homozygous AA, heterozygous AG, and homozygous GG carrier patients was 89, 89, and 94 respectively there was no statistically significant effect of rs 2228570 on systolic and diastolic blood pressure.

EFFECT OF POLYMORPHISM RS2228570 ON VIT D LEVEL

As shown in table 5, the mean plasma level of Vit D was higher in homozygous GG carriers (9.8), and lower in homozygous AA carriers (4.4). The difference was statistically significant in the plasma level of Vit D between AA carriers and AG carriers (P value was <0.028), (Fig.2). Although the mean Vit D level in GG carrier was higher than that of AG and AA carriers but it is difficult to decide whether the difference is statistically significant or not because we have only two patients carry GG allele.

DISCUSSION

The most frequent allele was A (67%) while the most frequent genotype was AG (61%). G allele has minor frequency 33% and GG genotype was 2% (p=0.8). There have been numerous studies looking at correlations between the rs2228570 variant and EH [20]. However, the findings have been contentious and inconclusive. In a study in Morocco, Errouagui and coauthors [21] studied 177 patients with hypertension and 222 normotensive persons of both genders and discovered that rs 2228570 polymorphism was related to AH in genotypes that are codominant, dominant, or recessive. The

frequency of the GG genotype was significantly lower in hypertension patients than in controls (P=0.002). The average vitamin D concentrations of patients with the AA, AG, and GG genotypes were "28.06±10.57, 29.04±11.97", and "26.40±19.15 ng/mL", respectively. Nevertheless, there were no statistically significant variations in vitamin D levels between AA and AG genotype patients (P=0.6463) or those with AA and GG (P=0.0767) [21]. In a prospective study by Wang et al. [22] recruited 1,211 Caucasian American men. Hypertension affected 695 of the patients. The vast majority of hypertensive people with a VDR gene polymorphism and the prevalence of Fok I polymorphism was found in 885 subjects. Only the recessive model revealed a link between the VDR rs2228570 and the risk for hypertension. In model 2, the (HR) multivariate hazard ratio for the occurrence of hypertension was associated with the GG genotype. Patients with the GG genotype were more likely than those with the AG and AA genotypes to have an increased risk of hypertension in relation to 25 (OH) D concentration [22]. In an Italian investigation, Cottone and colleagues recruited 72 control volunteers of both sexes and 71 patients with essential hypertension. AA, AG, and GG genotype frequencies were 50.7%, 42.3%, and 7.0%, in patients with hypertension, respectively, and 40.3%, 40.3%, and 40.0%, respectively, in participants in good health 50.0% and 9.7%, respectively. Patients with hypertension had frequencies of alleles of A and G of 71.8% and 28.2%, respectively, while normotensive people had allelic frequencies of 65.3% and 34.7%, respectively. All three Fok I polymorphism genotypes had distinct diastolic blood pressures (P=0.018). Diastolic blood pressure was greater in GG genotype patients compared to AG genotype patients (P=0.002) 25 (OH) D levels were shown to be negatively correlated with heartbeat blood pressure, and this association was significant statistically. Among patients with the AG genotype (P=0.035). No particular genotype or allele was associated with hypertension. Additionally, no correlation between the Fok I polymorphism and RP activity was found [23]. Our results indicate the mean plasma level of Vit.D was higher in homozygous GG carriers (9.8), and lower in homozygous AA carriers (4.4). There was a statistically significant difference in the plasma level of Vit D between AA carriers and AG carriers (P value was <0.028) Although the mean Vit D level in GG carrier was higher than that of AG and AA carriers but it is difficult to decide whether the difference is statistically significant or not because we have only two patients carry GG allele and thereby there is no statistically significant effect of VDR rs2228570 on SBP and DBP (p=0.6) and responsiveness to valsartan with 45.5% were considered as responders and 54.4% as non-responders. This may be either because of the small size of the samples in this study or a potent indication of the absence of association between this variant and EH in the Iraqi population. There is no clear explanation for why the rs2228570 variation is linked to hypertension. However, it has been proposed that the rs2228570 polymorphism is brought on by T to C change that causes a frameshift in translation marked by the creation of a truncated 424-amino-acid protein by extending the open reading frame to the next start codon (ATG) "ATG encoded methionine (M1 form)" was found in 427 amino acid protein's G allele, whereas "ACG encoded methionine (M4 form)" was found in the A allele. It is believed that the shortened protein in people with the AA genotype enhances the production of renin and angiotensin II, which in turn helps to develop essential hypertension [16].

CONCLUSIONS

Our research is the first of its kind in Iraq that investigates allele and genotype frequency in Iraqi patients with essential hypertension. Our result showed the most common allele for rs2228570 was the A allele 67% while the most frequent genotype was AG (61%), frequency of another genotype AA and GG were 37% and 2% respectively. Vit D receptor gene polymorphism rs2228570 was related to vit D level and there was no statistically significant effect on systolic and diastolic blood pressure.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Aseel R. Jabir
University of Al-Qadisiyah
University neighborhood, Al-Qadisiyah, Iraq
e-mail: aseelghufran9@gmail.com

ORCID AND CONTRIBUTIONSHIP
Aseel R. Jabir: 0009-0000-4429-0300 A B D
Hussein A. Saheb: 0000-0002-0137-8932 B C
Bassim I. Mohammad: 0000-0001-6732-5940 B C D
Ahmed M. Sultan: 0000-0001-6819-0208 C D
Sinaa Abdul Amir Kadhim: 0000-0001-9375-5581 D E
Asma A. Swadi:0000-0002-7679-1596 D E F

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

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ORIGINAL ARTICLE

CONTENTS

Evaluation and treatment of celiac disease in the central and south of Iraq

Shathel Khalaf Noman[1], Mahmood Jawad[2], Mohammed Rasool[3], Samir Jasim[2]

[1]DEPARTMENT OF PHARMACOLOGY, COLLEGE OF PHARMACY, TIKRIT UNIVERSITY, TIKRIT, IRAQ

[2]DEPARTMENT OF PHARMACY, AL-ZAHRAWI UNIVERSITY COLLEGE, KARBALA, IRAQ

[3]DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF KERBALA, KERBALA, IRAQ

ABSTRACT

Aim: To estimate the differences between patients with celiac disease based on symptoms, diagnosis, treatment, and follow-up.

Materials and Methods: A retrospective cross-sectional study carried out between July 1, 2022 and April 2023, enrolling 200 patients from different provinces of central and south Iraq with Celia disease, whose diagnosis depended on a specialized physician according to WHO guidelines with long-term follow-up. Participants were following up for three to six months in private clinics. Survey was written in English, and the questionnaire form contains 13 fields divided into three sections. Diagnosis of Celia before and after treatment parameters: Tissue Transglutaminase Antibody, IgG, Serum (tTg-Ig G), and tTg-IgA levels the fourth part included a glutin-free diet and symptomatic treatment.

Results: Females and ages below 20 were most affected. 176(88%) patients had detectable tTG levels; after 3 months, 72(36.0%) patients had an increase in their body weight but less than 5 kg, while 14(7.0%) of the patients showed an increase of more than 5 kg. But after 6 months, 73(36.5%) patients had an increase in their body weight less than 5 kg, while 45(22.5%) of patients showed an increase of more than 5 kg.

Conclusions: Celiac patient profile in central Iraq is not different from that in other parts of the world, with typical patient being female and under 30 years of age. The study highlighted to a certain degree that a gluten-free diet can have a modest and promising positive impact on BMI in some patients.

KEY WORDS: gluten, celiac disease, and tissue transglutaminase antibody

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INTRODUCTION

Coeliac disease (CD) is a persistent immune-mediated enteropathy affecting the small intestine, which is triggered by the consumption of gluten in those who possess genetic susceptibility, and the genetic vulnerability plays a significant impact, as a clear majority of patients >95% exhibit the HLA-DQ2 gene, whereas approximately 5% exhibit the HLA-DQ8 gene [1]. Coeliac disease is a prevalent hereditary condition worldwide and it affects about 1 in 100 persons in the world. The prevalence of the disease is higher in females compared to males, with a ratio of 3 to 1 [2]. Despite of the clear rise in the occurrence of sickness, one frequently encountered obstacle in the process of diagnosis is occasionally associated with the variability observed in the manifestation of certain features across different patients. Clinically CD showed a highly diverse range of symptoms, from the standard (gastrointestinal) aspects to unusual (extra intestinal) ones [3]. Celiac disease is characterized by a diverse range of clinical manifestations related to gluten, specific antibodies, and the presence of HLA-DQ2 or HLA-DQ8. This condition is associated with a distinct pattern of intestinal damage, including inflammatory cell infiltration, crypt hyperplasia, and ultimately villous atrophy. Following a gluten-free diet (GFD) can lead to both clinical and serologic remission, which are considered key features of CD [4]. In light of the multiple extraintestinal symptoms of the disease, celiac disease may be more appropriately viewed as a systemic issue than only a gastrointestinal disorder [5]. Extraintestinal symptoms are typical in both children and adults. Iron deficiency microcytic anaemia is a prevalent condition that can be detected in approximately 40% of patients (by the underlying factor causing iron malabsorption or chronic inflammation) [6] or, less frequently, macrocytic anemia brought on by a vitamin B12 and/or folic acid deficiency (more common in Europe than the US). Affected calcium and vitamin D3 absorption is the occurrence of alterations

in bone mineral density, such as osteopenia or osteoporosis, has been observed in approximately 70% of patients upon diagnosis [7]. The presence of growth retardation and small stature in children may indicate a possible underlying CD. Defects in the enamel of the teeth and aphthous stomatitis (seen in roughly 20% of CD patients who are undiagnosed) are additional symptoms [8]. The most frequent clinical signs of CD typically involve recurrent episodes of disturbed bowel habits (diarrhea and/or constipation), presence of gastro-esophageal reflux (GERD), and weight loss [9]. The clinical signs and disease-specific antibodies are used to make a diagnosis. The gold standard for confirming a diagnosis is duodenal mucosal biopsies. Before following a gluten free diet, the serology and biopsy should both be completed (GFD) [10]. HLADQ2/DQ8 genotyping is possible in certain situations. The HLA gene DQ2 genotype predominates among celiac patients in Iraq [11]. The detection of celiac disease can be achieved through the use of anti-endomysial (EMA) antibodies of the immunoglobulin A (IgA) type, which exhibit a sensitivity of 90% and a specificity of 99% in patients with villous atrophy. Initial reports claimed that anti-transglutaminase antibody serology had a higher specificity of >90% and a sensitivity of 99% than other methods. However, it is currently believed to share traits with anti-endomysial antibodies [12].

AIM

The aim of the study is to determine the variability within a specific population-based cohort of individuals diagnosed with celiac disease with regard to age, gender, haemoglobin, body weight, diagnosis tests, treatment, and follow-up.

MATERIALS AND METHODS

A retrospective cross section study carried out between July 1, 2022 and April 2023 that enrolling 250 patients from different provinces of central and south Iraq (Babylon, Karbala, Al Najaf, Al Muthanna, and Diwaniya) with celiac disease that diagnosis depended on specialized physician according to WHO guidelines, and excluded 50 patients due to the lack of diagnostic evidence, insufficient lab results and inability to follow up for 6 months with them for different causes, and the study was completed on 200 patients in detail and with long-term follow-up. The participants were following up for three and six months in private clinics. The research form contained 13 fields divided into five sections. The first section contains social information such as age, gender, and education. The second part includes signs and symptoms, which included epigastric pain, loose motion, oral ulcers, weight loss, severe pallor, nausea, vomiting, diarrhea, anemia, and anorexia and other chronic disease. The third part consists of diagnosis which included the body weight, hemoglobin, tissue transglutaminase antibody, IgG, serum tTg-Ig G, and tTg-IgA levels. The fourth part included gluten free diet (GFD) and symptomatic treatment. In addition, for the final part it included the patient follow up after 3 months and 6 months of treatment and the responsiveness of the approach. After that, we collect the obtained data in Microsoft Excel program tables and then statistically analyzed it as shown in the results using IBM SPSS Statistics version 26.

STATISTICAL ANALYSIS

The data analysis was conducted using SPSS® version 26, a statistical tool developed by IBM Inc. in Chicago, IL, USA. The standard deviation was used to express the numerical variables. When the Kolmogorov-Smirnov test failed to reveal a normal distribution, the variables were examined using the nonparametric Kruskal-Wallis test to evaluate the mean differences between groups. Bivariate correlation analysis was also carried out to evaluate the relationship between markers. Statistics were deemed significant at $P < 0.05$.

RESULTS

During the study period, 200 patients with celiac disease were included, 44 (22.0%) were males and 156 (78.0%) were females (χ^2=42.440, P-value=0.000). The mode age of the participants was in age group of 20-29 years, where was 76 (38.0%) of participants (χ^2=62.72, P-value=0.000), however, 119 (59.5%) of patients with university education was consider significant when compared with other educations (χ^2=150.52, P-value=0.000), 75 (37.5%) patients had more than three symptoms (χ^2=58.75, P-value=0.000). On other hands, the 107 (53.5%) patients had low hemoglobin level; also, the frequency and percentage of the subtypes are shown in Table 1.

ASSOCIATION OF DETECTABLE TTGG IGA AND TTG-IG G WITH GENDER, EDUCATION, HB, AND BODY WEIGHT

Out of the total sample size of a 176 patients, 88% exhibited detectable tTg levels, while the remaining 12% showed undetected tTg levels. The current study reveals a notable negative correlation between the

Table 1. Frequencies and percentages of the variant's subtypes in the study

		Frequency	Percent	SD	Chi-Square	P value
Age	<20	45	22.5	1.623	42.440	0.000
	20-29	76	38.0			
	40-49	15	7.5			
	≥ 50	64	32.0			
	Total	200	100.0			
Gender	Male	44	22.0	0.415	62.72	0.000
	Female	156	78.0			
	Total	200	100.0			
Education	Illiteracy	7	3.5	0.811	150.52	0.000
	Primary	20	10.0			
	Secondary	54	27.0			
	University	119	59.5			
	Total	200	100.0			
	Total	200	100.0			
Total no. of symptoms	None	12	6.0	1.240	58.75	0.000
	One	23	11.5			
	Two	44	22.0			
	Three	46	23.0			
	More than three	75	37.5			
	Total	200	100.0			
Hb	Normal	93	46.5	0.50003	0.98	0.322
	Low	107	53.5			
	Total	200	100.0			

	tTg-Ig G	tTg-Ig A	Gender	education	Hb

measured tTgG IgA and tTg-IgG and hemoglobin levels. This indicates that when the level of HB is high or close to normal in individuals with celiac disease, there is a corresponding decrease in these stereological tests and vice versa. This pattern is also observed in patients with levels of education, also these seen with gender that may be due to most cases in present study from female patients (Table 2).

The statistical analysis using the chi-square test yielded a $\chi 2$ value of 115.52, with a corresponding P-value of 0.000, indicating a significant association between tTg levels and the patient group. At the time of the study, the average duration of adherence to a gluten-free diet ranged from 3 to 6 months. After 3 months, 72 (36.0%) patients had an increase in body weight, but less than 5 kg, and 14 (7.0%) patients had an increase in body weight of more than 5 kg. However, after 6 month, 73 (36.5%) patients with increased in their body weight less than 5 kg while 45 (22.5%) of the patients showed increased more than 5 kg (Table 3).

DISCUSSION

An autoimmune enteropathy caused by food gluten sensitivity in people with certain genetic predispositions is called celiac disease [13]. It covers a wide range, from overt malabsorption or common gastrointestinal symptoms to disorders that are clinically quiet [14]. Due to the advancement of diagnostic techniques for identifying CD, the loss of iron in the intestine enterocytes, malabsorption of daily iron, and very infrequently gastrointestinal hemorrhage may cause the pathogenesis of CD [15]. Studies using small-bowel biopsies and serologic assays in patients referred for IDA investigation have revealed CD in 1.8 percent to 14.6 percent of patients [16]. This study examines the prevalence of celiac disease in patients using a very sensitive screening test (the tTG antibody test). Our study showed that the Iraqi patients of with celiac disease with symptoms in the Center and South area of Iraq had 88.0% to tTG (IgA and IgG) positive titer. On other hands, our study showed there was a significant gender difference between the participants; the majority of them, specifically 156 (78.0%), were female

Table 2. Frequencies of the serological tests and gender, education, and Hb

		tTg-Ig G	tTg-Ig A	Gender	Education	Hb
tTg-Ig G	Pearson Correlation	-	.148*	-0.354**	-0.134*	-0.357**
	Sig. (2-tailed)	-	0.087	0.001	0.016	0.006
	N	200	200	200	200	200
tTg-Ig A	Pearson Correlation	0.148*	-	-0.414**	-0.139*	-0.288**
	Sig. (2-tailed)	0.087	-	0.011	0.013	0.017**
	N	200	200	200	200	200

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 3. Frequencies and percentages of the serological and follow-up in the study

		Frequency	Percent	SD	Chi-Square	P value
tTg-Ig G	Negative	24	12.0	0.326	115.52	0.000
	Positive	176	88.0			
	Total	200	100.0			
tTg-Ig A	Negative	24	12.0	0.326	115.52	0.000
	Positive	176	88.0			
	Total	200	100.0			
Follow up 3 months						
Weight < 5kg	Yes	72	36.0	0.483	15.68	0.000
	No	128	64.0			
	Total	200	100.0			
Weight > 5kg	Yes	14	7.0	0.256	147.92	0.000
	No	186	93.0			
	Total	200	100.0			
Follow up 6 months						
Weight < 5kg	Yes	73	36.5	0.483	14.58	0.000
	No	127	63.5			
	Total	200	100.0			
Weight > 5kg	Yes	45	22.5	0.419	60.5	0.000
	No	155	77.5			
	Total	200	100.0			

while only 44 (22.0%) were male. This is in accordance with general established research stating that more women get diagnosed with celiac disease than men [2, 17]. This may be due to the fact that women are thought to be predisposed to autoimmune disease and the reason for which is not yet completely understood [18]. In regards to symptoms, 75 (37.5%) of the participant reported having more than 3 symptoms at the time of diagnosis while the rest of the patients 125 (62.5%) reported having three or less symptoms. Recent research has found that the number of patients presenting with mild symptoms has been increasing in the recent years [19], the most common of these symptoms is chronic diarrhoea, weight loss and abdominal distension. Also, a significant portion of the participants 107 (53.5%) had low haemoglobin levels indicating a tangible correlation between anaemia and celiac disease. Since iron is a vital micronutrient, a lack of it in CD is typically caused by malabsorption owing to destruction to the intestinal mucosa's villi. However, the diminished expression of many regulatory proteins may also contribute to iron shortage in CD [20]. Serology tests of the patients showed that 176(88%) patients had detectable tTG levels and 24(12%) patients had undetectable tTG levels. This demonstrates the accuracy and sensitivity of these tests making them an important and significant biomarker in the diagnosis process, a process which previously relayed on histological assessment of intestinal mucosa but since the 1980s of the last century serological tests became the first step when there is a suspicion of celiac disease [21]. At the

time of the study, a gluten free diet lasted three to six months on average. After 3 months, 72 (36.0%) patients with an increase in their body weight but less than 5 kg while 14 (7.0%) of the patients showed increased more than 5 kg. However, after 6 month, 73 (36.5%) patients with increased in their body weight less than 5 kg while 45 (22.5%) of the patients showed increased more than 5 kg. This data illustrates that a significant portion representing the majority did not have an increase in their body weight. A possible explanation for this might be refectory celiac disease due to non-completely adherence to diet (GFD) or they use food that they think is free of gluten, as in some cereals, biscuits, and pastries, but they contain gluten. Even though it's uncommon, RCD should be considered in those with a confirmed diagnosis of CD who don't improve after trying a rigorous gluten-free diet, especially if they have severe weight loss or no weight gain [22]. For the remaining patients who did gain weight the majority of them experienced it on a small scale, that is <5 kg this could be an indication of the ineffectiveness of the gluten free diet in improving the clinical manifestation of weight loss in celiac patients but it can also be attributed to the relatively short time frame in which the follow ups occurred. This proposes the idea that the slight increase in weight was a reflection of the duration of the study rather than the effectiveness of the GFD diet. To further support this claim, other studies with a longer duration of follow-ups showed better results in regards to weight gain an example for which, when compared to the control group, A prospective research with 39 CD patients and 39 controls the study shown that individuals who were initially diagnosed with a body mass index (BMI) within the normal range 18.5-24.9 Kg/m2 had weight gain over time. Conversely, it was seen that 50% of patients who had an underweight BMI 18.5 Kg/m2 at diagnosis transitioned to the normal weight category following a two-year follow-up period [23]. Furthermore, a variety of retrospective studies involving 679 individuals diagnosed with celiac disease and adhering to a gluten-free diet (GFD) revealed that 22% of overweight patients experienced weight gain following the initiation of a GFD. Additionally, 15.8% of these individuals transitioned from a normal or low body mass index (BMI) category to an overweight classification BMI>25 kg/m2 subsequent to commencing a GFD. The duration of these studies ranged from 1 to 345 months, with a mean duration of 39.5 months. In general, there was a significant increase in the average body mass index (BMI) following adherence to a gluten-free diet (GFD). Specifically, among the participants who initially had a BMI within the normal range, 21.8 percent had weight gain, resulting in a BMI increase over two units [24].

CONCLUSIONS

The study data demonstrated that the celiac patient profile in central Iraq is not different to that in other parts of the world with the typical patient being female and under 30 years of age. More importantly the study highlighted to a certain degree that gluten free diet can have a modest and promising positive impact on BMI in some patients. However, the total majority did not gain any weight in the first place and for the ones that did experience and improvement in weight they gained less than 5 kg total. This can lead to the understanding that for patients of celiac disease who are following the gluten free diet, it might take a significant amount of time to see benefits and improvements at least regarding weight. A possible limitation of this study was the personal adherence of patients to GFD.

RECOMMENDATIONS

Since GFD can be beneficial to a significant portion of celiac disease patients in Iraq, it is safe to say that is should be considered for all patients as part of the main management strategy. Also, efforts should be made by government and even private stores or markets to make gluten free options more available and accessible. Finally, all patients should be urged to adhere to the diet in spite of the social, economic and personal burdens that may accompany it since, to this day it remains the only true management method. Or if can introduce drugs that decrease the absorption of gluten from GIT.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Mahmood Mahmood

Al-Zahrawi University College

G499+JPR Karbala, Iraq

e-mail: mahmood@g.alzahu.edu.iq

ORCID AND CONTRIBUTIONSHIP
Shathel Khalaf Noman: 0000-0002-7065-6924 B C
Mahmood Jawad: 0000-0001-6542-7230 A F
Mohammed Rasool: 0000-0003-0992-320X C D
Samir Jasim: 0009-0000-0396-8517 E

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

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ORIGINAL ARTICLE

CONTENTS

Brain morphometry and short-term stroke outcome

Dmytro Smolko, Roman Bartiuk, Lesia Zheliba, Yaroslava Marunkevych, Olga Gordiichuk, Natalia Starynets, Iryna Olkhova

VINNYTSIA NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

Aim: The aim of the research was to investigate associations between brain morphometric changes and short-term stroke outcome.

Materials and Methods: In this study, 294 patients with acute stroke were enrolled. All participants underwent magnetic resonance imaging (MRI) and computed tomography (CT) assessment as well as clinical-neurological and cognitive testing.

Results: In the multivariable regression analysis, bicaudate index (OR = 1.3; 95 % CI 1.1 - 1.7, p=0.018) and ventricular index (OR = 0.7; CI 0.5 - 0.9, p=0.005) were associated with an unfavourable short-term stroke outcome. The univariable regression analysis revealed significant associations between mini-mental state examination scale score (MMSE) and width of the longitudinal cerebral fissure in the anterior part of the frontal lobes (FI) (b -0.8, 95% CI -1.6 – -0.1, p=0.037) as well as width of the cerebral fissure in the area of the skull vault (SW) (b -0.9, 95% CI -1.8 – -0.1, p=0.023). In the multivariable regression model bicaudate index was associated with MMSE score (b coefficient (b) = -1.2; 95 % CI -2.1 – -0.3, p = 0.011).

Conclusions: our results show that altered brain morphometric indices are associated with unfavourable short-term stroke outcome and cognitive decline.

KEY WORDS: stroke, MMSE, mRS, brain morphometry, bicaudate index

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INTRODUCTION

Stroke is the leading cause of long-term disability and the second most common cause of death on the globe. The estimated global cost of stroke is over US $ 891 billion (1.12% of the global GDP) [1]. The clinical outcome of stroke patients depends on multiple factors [2], however the data on the brain morphometric changes beyond stroke lesion and their relationship to stroke outcome are sparse.

Vascular dementia is the second most common cause of dementia affecting the elderly. [3]. Hence, understanding the relationship between brain morphometrics, stroke outcome and cognitive decline can be useful for early diagnostics and prognostic purposes.

Brain atrophy reflects a final common pathway for pathological processes in both cerebrovascular and neurodegenerative disorders [4, 5]. Therefore, more precise, and thorough measurements of the brain morphology can help formulating better prognosis and prevention strategies after stroke.

We tested a hypothesis that altered brain ventricular indices and cortical sulci span are predictors of unfavourable short-term stroke outcome at discharge.

AIM

The aim of the research was to investigate associations between brain morphometric changes and short-term stroke outcome.

MATERIALS AND METHODS

SUBJECTS

This research is a part of a prospective cohort study "Diagnosis and predictive value of cerebral small vessel disease in the acute phase of cerebral stroke", which was conducted from December 2016 to December 2021 and was based at specialised stroke department (Stroke Unit) № 22 of the Vinnytsia Regional Psychoneurological Hospital named after acad. O.I. Yushchenko. Between December 2016 and December 2019 294 consecutive patients with acute stroke were recruited. We collected clinical, neurological and neuroimaging data during the period of hospitalization of the patients. In face-to-face interviews with patients or their caregivers we collected a variety of data, which is described below. This information was cross-referenced with primary care records

and medical records during follow-up. The average age of the participants was 61.9 ± 10.1 years, of which 179 were men and 115 women. The follow-up period was 9.4 ± 4.2 days. The study was approved by the local ethics committee (Protocol № 9 of November 14, 2016, and Protocol №4 of May 18, 2023). All patients signed informed consent before the enrolment procedure.

The main criteria for the selection of patients were confirmed diagnosis of stroke, proper quality of neuroimaging scans and obtained informed consent to participate in the study. The exclusion criteria were: age under 18 years old, insufficient quality of neuroimaging data or presence of neuroimaging artifacts, neuroimaging evidence of brain lesions of non-vascular origin.

IMAGE ACQUISITION

120 patients underwent MRI, 174 - CT. Some of the subjects were imaged with either MRI or CT, some of them - with MRI and CT both. MRI was performed on a Philips Achieva 1.5T. The brain scanning protocol included the following whole brain scans: T1-weighted, T2-weighted, FRAIR and DWI sequences, slice thickness was 3.5-5 mm. CT was performed on a General Electric CT/e (Italy) with a tomographic slices of 3-7 mm.

MEASUREMENTS OF THE BRAIN MORPHOMETRY

We collected MRI and CT data of each patient and visually assessed such brain morphometrics as Evans index, third ventricle index, fourth ventricle index, ventricular index, Shlatenbrandt-Nurenberger index, bicaudate index, cella media index (also known as Schiersmann's index), Huckman number using computer software "The Horos Project". All ventricular indices were multiplied by 100 for better representation. We also measured cortical areas: FI, the width of the right and left insular cisterns and their sum (ICR, ICL, ICRL), SW and the maximum width of the cerebellar fissure (CFW) [6].

CLINICAL ASSESSMENT

The demographics and the risk factors were collected: age, sex, body mass index (BMI), Charlson comorbidity index [7], previous stroke, smoking, hypercholesterolemia. NIHSS score [8], modified Rankin scale (mRS) score [9], Barthel index score (BI) [10], Glasgow coma scale (GCS) [11], MMSE score [12] were assessed on admission and at discharge as part of the clinical workup. Stroke subtypes were determined based on the TOAST criteria [13]. The short-term functional outcome was assessed with the mRS and BI at discharge. Also, the functional outcome was measured with the mRS at discharge as favourable outcome (mRS ≤ 2) or unfavourable outcome (mRS > 2) [14]. We defined short-term period as a time from initial presentation to discharge; mean time of hospitalisation was 9.4 ± 4.2 days.

STATISTICAL ANALYSIS

Both univariable and multivariable regression models were used to determine the associations between morphometric data and stroke outcome. The results are shown as odds ratio (OR) and 95% confidence intervals (CI) or as a b-coefficient and 95% CI in case of linear regression. Continuous variables were presented as mean ± standard deviations (SD) or median and interquartile range (ICR). A p value < 0.05 was considered statistically significant. A two groups comparison of brain morphometrics between patients with favourable and unfavourable stroke outcome (functional dependence, mRS >2) was performed by Student's *t*-test in normal distribution or Mann–Whitney *U* test if the variables were not normally distributed. Categorical variables were presented as percentages and were compared with Pearson's chi square test or Fisher's exact test (if number of observations was <5). Statistical analysis was performed by The jamovi project (2022). Jamovi (Version 2.3) [Computer Software]. Sydney, Australia.

RESULTS

In the table 1 clinical and demographical data of the total population is shown. To reveal their associations with stroke outcome along with the indices we build a multifactorial regression model, where most of these predictors as well as brain indices (step by step) were included.

According to the univariable regression analysis, the third ventricle index, Shlatenbrandt-Nurenberger index, bicaudate index, FI, SW significantly associated with poorer BI at discharge (b: -2.5; (95 % CI -4.6 - -0.3, p = 0.024), 0.5; (95 % CI 0.1 - 0.9, p = 0.022), -1.7; (95 % CI -2.8 - -0.6, p = 0.002), -3.1; (95 % CI -5.6 - -0.6, p = 0.014), -2.9; (95 % CI -5.5 - -0.4, p = 0.026) respectively).

After adjusting for age and sex, in the multivariable regression analysis significant associations were found between bicaudate index and BI at discharge (b = -1.3; 95% CI -2.4 – -0.1, p=0.035).

In the next multivariable regression model, adjusted for age, sex, history of smoking, comorbidity index, body mass index, history of stroke, hyperlipidemia, presence of complications, index stroke severity by NIHSS, significant association was found between ventricular index and BI at discharge (b = 1.4; 95 % CI

Table 1. Clinical and demographic characteristics of the participants, N=294

Characteristics	
Age	61,9 ± 10,1
Sex male female	 179 (61%) 115 (39%)
TOAST classification, n (%) large artery atherosclerosis cardioembolism small vessel occlusion other determined stroke undetermined stroke	134 (48 %) 71 (25 %) 42 (15 %) 3 (1 %) 29 (11 %)
Period of hospitalisation, days	9,4 ± 4,2
Smoking	66 (29%)
History of previous stroke	71 (24%)
Hypertension	267 (92%)
Ischemic heart disease	34 (12 %)
Atrial fibrillation	96 (33 %)
Diabetes Mellitus	47 (16%)
Charlson comorbidity index	Median 2 (IQR 1 – 3)
Complications	84 (29 %)
BMI	29,7 ± 4,8
Hypercholesterolemia	167 (61 %)
Stroke size, ml	38,0 ± 69,6
NIHSS on admission	11 (8 – 16)
NIHSS at discharge	5 (2 – 11)
mRS on admission	4 (4 – 5)
mRS at discharge	3 (1 – 4)
GCS on admission	14 (13 – 15)
GCS at discharge	15 (14 – 15)
BI at discharge	75 (40 – 100)
MMSE	18,7 ± 10,5

0.2 - 2.6, p = 0.024). Also, significant associations were found between index stroke severity by NIHSS and BI at discharge (b = 1.4; 95 % CI 0.2 - 2.6, p = 0.024), comorbidities and BI at discharge (b = -4.0; 95 % CI –7.3 - -0.7, p = 0.019), presence of complications and BI at discharge (b = -19.3; 95 % CI –27.2 - -11.4, p < 0.001). In this model, third ventricle index, bicaudate index and Evans index showed near-marginal significance (p < 0.1).

The univariable regression analysis revealed a significant association between MMSE and FI (b = -0.8; 95% CI -1.6 – -0.1, p=0.037) as well as SW (b = -0.9; 95% CI -1.8 – -0.1, p=0,023), ICRL was nearly significant (b = -0.2; 95% CI -0.5 – 0.03, p=0.084).

In the multivariable linear regression model, adjusted for age, sex, NIHSS on admission and the brain indices, only bicaudate index was associated with MMSE score (b = -1.2; 95 % CI -2.1 – -0.3, p = 0.011) along with NIHSS on admission (b = -1.2; 95 % CI -1.4 – -1.0, p < 0.001).

In table 2 and fig. 1, bicaudate index, ventricular index, FI and SW significantly differ in case of favourable (mRS 0 – 2, functional independence) vs unfavourable (mRS 3 – 6, functional dependence) stroke outcome.

Further, multifactorial regression analysis was performed, adjusted for age, sex and NIHSS on admission along with all morphometric indices to evaluate their impact on the unfavourable stroke outcome. Both crude and adjusted odds ratio are shown in table 3.

The next multifactorial regression model was made using only ventricular indices, adjusted for each other's, age and sex, as shown in fig. 2.

DISCUSSION

In this study we investigated brain morphological alterations and their associations with short-term stroke outcome. Our findings confirm that brain morphomet-

Table 2. Comparisons of brain morphometrics between patients with favourable and unfavourable stroke outcome (functional dependence, mRS >2)

Brain measurement	favourable outcome Mean ± SD	unfavourable outcome Mean ± SD
Evans index	26.8 ± 3.9	27.5 ± 3.9
The third ventricle index	4.9 ± 1.7	5.2 ± 1.8
Shlatenbrandt- Nurenberger index	23.6 ± 9.3	21.7 ± 8.7
The fourth ventricle index	12.5 ± 1.6	12.5 ± 2.0
Bicaudate index	14.8 ± 3.3	16.0 ± 3.6**
Ventricular index	16.8 ± 2.6	16.2 ± 2.5*
Cella media index (Schiersmann's index)	5.3 ± 1.2	5.1 ± 1.2
Huckman number	55.9 ± 8.7	57.9 ± 9.8
FI	5.3 ± 1.5	5.7 ± 1.6*
ICR	7.0 ± 2.4	7.4 ± 2.8
ICL	7.7 ± 2.7	7.8 ± 2.5
ICRL	14.7 ± 4.5	15.2 ± 4.9
SW	4.1 ± 1.4	4.5 ± 1.6*
CFW	2.5 ± 1.1	2.6 ± 1.2

* p<0,05, **p<0,01.

Table 3. Brain morphometric indices, reflecting its impact on the unfavourable stroke outcome (functional dependence, mRS >2) at discharge.

Brain measurement	Crude OR (95% CI, p)	Adjusted OR (95% CI, p)
Evans index	1.1 (1.0-1.2, p=0.118)	0.8 (0.6-1.0, p=0.099)
The third ventricle index	1.1 (1.00-1.3, p=0.061)	1.4 (0.9-2.4, p=0.166)
Shlatenbrandt- Nurenberger index	0.98 (0.95-1.00, p=0.072)	1.1 (0.99-1.2, p=0.080)
The fourth ventricle index	0.99 (0.9-1.1, p=0.922)	0.9 (0.8-1.1, p=0.295)
Bicaudate index	1.1 (1.0-1.2, p=0.003)	1.3 (1.1-1.7, p=0.018)
Ventricular index	0.9 (0.8-1.0, p=0.073)	0.7 (0.5-0.9, p=0.005)
Cella media index (Schiersmann's index)	0.9 (0.7-1.1, p=0.298)	1.0 (0.7-1.6, p=0.858)
Huckman number	1.0 (1.00-1.1, p=0.066)	0.9 (0.8-1.1, p=0.332)
FI, 1 mm	1.2 (1.0-1.4, p=0.024)	0.97 (0.7-1.3, p=0.840)
ICR, 1 mm	1.1 (1.0-1.2, p=0.219)	0.7 (0.1-3.4, p=0.765)
ICL, 1 mm	1.0 (0.9-1.1, p=0.605)	0.6 (0.1-3.0, p=0.705)
ICRL, 1 mm	1.0 (1.0-1.1, p=0.288)	1.6 (0.3-16.2, p=0.760)
SW, 1 mm	1.2 (1.0-1.5, p=0.018)	1.2 (0.9-1.7, p=0.155)
CFW, 1 mm	1.1 (0.9-1.3, p=0.418)	1.3 (0.9-1.8, p=0.161)
Age, 10 years	1.4 (1.1-1.8, p=0.006)	1.1 (0.8-1.6, p=0.536)
Sex, female	1.0 (0.7-1.7, p=0.888)	1.1 (0.6-2.3, p=0.734)
NIHSS at admission, 10 points	1.3 (1.2-1.4, p<0.001)	1.4 (1.3-1.5, p<0.001)

rics are significantly correlated with short-term stroke consequences.

The results revealed that some morphometric indices deteriorate stroke severity during the time of hospitalisation. According to the univariable regression analysis, the third ventricle index, Shlatenbrandt-Nurenberger index, bicaudate index, FI, SW were significantly associated with poorer BI at discharge (b: -2.5; 0.5; -1.7; -3.1; -2.9; respectively). In multivariable regression analysis, bicaudate index (b = -1.3; 95% CI -2.4 – -0.1, p=0.035) and ventricular index (b = 1.4; 95 % CI 0.2 - 2.6, p = 0.024) were also significantly associated with poorer BI at discharge.

When we dichotomized stroke outcome as a favourable (mRS at discharge 0 – 2) and an unfavourable (mRS at discharge 3 – 6, – functional dependence), in the univariable regression analysis bicaudate index (OR = 1.1; 95 % CI 1.0 - 1.2, p=0.003), FI (OR = 1.2; 95 % CI 1.0 - 1.4,

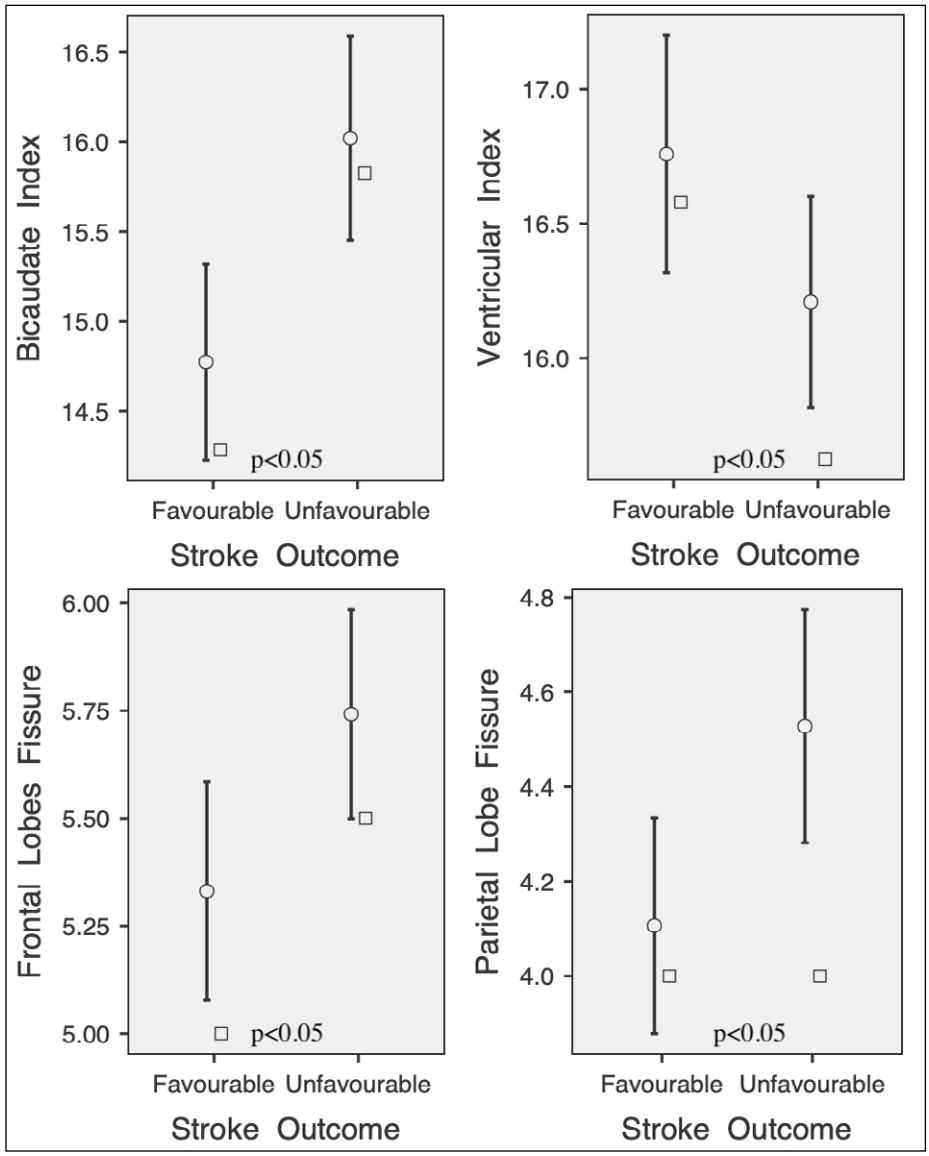

Fig.1. Significant differences in brain morphometrics depending on stroke outcome, o - mean (95 % CI), ▢ - median.

p=0.024), SW (OR = 1.2; 95 % CI 1.0 - 1.5, p=0.018) were associated with an unfavourable stroke outcome. In the multivariable regression model, adjusted for age, sex, NIHSS on admission, bicaudate index (OR = 1.3; 95 % CI 1.1 - 1.7, p=0.018) and ventricular index (OR = 0.7; CI 0.5 - 0.9, p=0.005) were associated with an unfavourable short-term stroke outcome.

There are several possible explanations for this: larger ventricular sizes reflect decreasing white matter volume, which may lead to a loss of functional connectivity. It can disrupt the white matter tracts or U-fibers that mediate cortical–cortical or cortical–subcortical connections. Besides, some research revealed that local white matter lesions may influence the grey matter in remote areas [15]. Hence, altered brain morphometric indices can be linked with unfavourable stroke outcome by impaired recovery of lost functions.

The indices enlargement reflects end-term lesions of the brain parenchyma and imply a variety of pathophysiological aspects, which unfavourably affect clinical consequences of stroke. Acute phase of stroke require comprehensive vascular and neural mechanisms to cope with ischemia, but presence of atrophy can deteriorate that through neuronal disintegration. Promotion of new brain cortical maps and networks is probably worsened due to disintegration of neuronal connections, which are seen at the macroanatomical level as altered cortical and ventricular morphometric parameters.

Over time, particular cortical areas can not only atrophy, but get thicker. Possible causality might occur due to the neuroplasticity mechanisms triggered by the stroke and occurring at a distance from the lesion [16]. Further investigation of the relationship between cerebral morphometric parameters and long-term motor and cognitive recovery after stroke can be of interest to test these hypotheses.

Lower MMSE score was significantly associated with

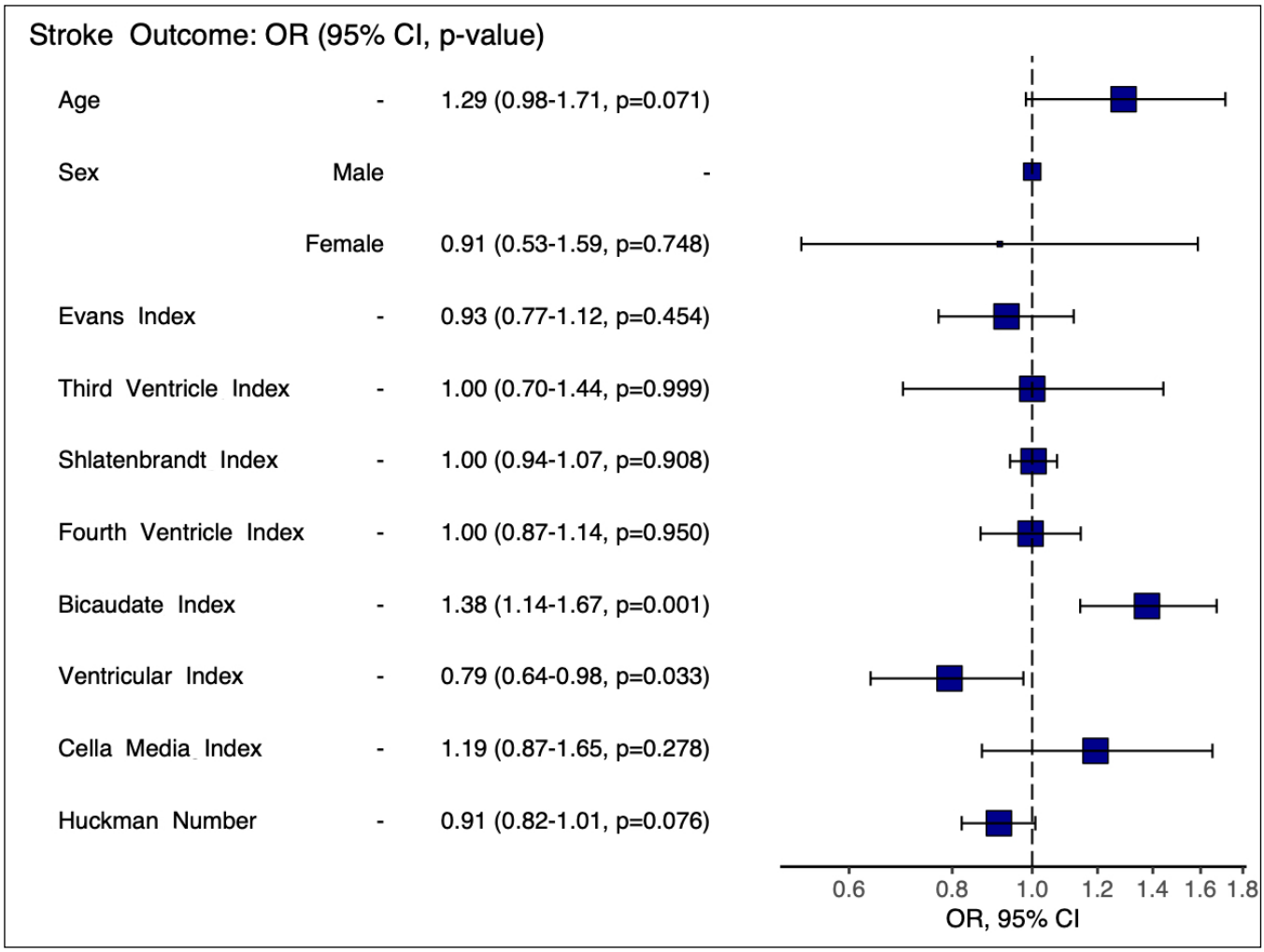

Fig.2. Forest-plot of ventricular indices associations with unfavourable stroke outcome at discharge.

altered cortical indices (cortical thinning) in the univariable regression analysis: FI (b -0.8, 95% CI -1.6 – -0.1, p=0.037) as well as SW (b -0.9, 95% CI -1.8 – -0.1, p=0.023), ICRL was nearly significant (b -0.2, 95% CI -0.5 - 0.03, p=0.084). In the multivariable regression model only bicaudate index was associated with MMSE score (b = -1.2; 95 % CI -2.1 – -0.3, p = 0.011) along with NIHSS on admission (b = -1.2; 95 % CI -1.4 – -1.0, p < 0.001). Other indices lost significance, suggesting that in an acute phase of stroke the lesion size and NIHSS severity on admission are key contributors to an acute cognitive deterioration. Some findings suggest that white matter damage that occurred due to cerebral small vessel disease promote atrophy (ventricular and sulci span enlargement), which, in turn, drives cognitive decline [17].

Neurobehavioral features of vascular dementia are slowed processing speed and executive dysfunction and emerged as the most prominent clinical features of white matter damage. With respect to cognitive slowing, the normal physiological function of myelin to increase axonal conduction velocity would be deteriorated [19]. Slowed processing speed can result from white matter degradation, which associated with ventricular system enlargement.

Many cognitive processes are realised by spatially distributed neural networks in the brain. The prefrontal and temporal cortex are a set of functionally connected regions that plays crucial roles in internal cognitive processing like working memory, attention and language, processing speed, autobiographical memory etc. [19, 20]. The parietal lobe is a heteromodal convergence zone of various brain networks that is central to realizing key cognitive operations across different levels of the neural processing hierarchy. These mental operations include lower level processes, such as spatial attention, as well as higher level processes that are distinctly elaborate in the human species, like semantic memory and modes of social exchange [21]. So, its damage can cause cognitive deterioration. Early injury of temporal lobes might be the reason of more serious cognitive decline [15], that is why measurement of the width of the insular cisterns and other cortical and subcortical regions might be useful for patients selection for early cognitive decline prevention, like acetylcholinesterase inhibitors etc., whether it is neurodegeneration or vascular origin. Hence, atrophy of cortical

areas, that are seen as altered aforementioned indices, can dramatically impair various cognitive functions, which is important to diagnose at the early stages.

For further investigations, measurements of ventricles sizes and cortical sulci span may be of interest, particularly for understanding how they impact long-term stroke outcome; as well as whether some brain morphometrics alterations associated with specific cognitive, functional, or behavioural changes. It is also necessarily to elicit how rehabilitation and other therapy can affect morphometric changes' progression in long-term perspective in stroke-survivors.

CONCLUSIONS

1. Brain morphometric alterations were associated with short-term stroke outcome in hospitalization period: in the multivariable regression analysis, bicaudate index (OR = 1.3; 95 % CI 1.1 - 1.7, p=0.018) and ventricular index (OR = 0.7; CI 0.5 - 0.9, p=0.005) were associated with an unfavourable short-term stroke outcome (functional dependence) and lower level of activities of daily living according to BI at discharge: bicaudate index (b = -1.3; 95% CI -2.4 – -0.1, p=0.035), ventricular index (b = 1.4; 95 % CI 0.2 - 2.6, p = 0.024).
2. Morphometric indices were associated with cognitive decline in acute phase of stroke: in the multivariable regression model bicaudate index was negatively associated with MMSE score (b = -1.2; 95 % CI -2.1 – -0.3, p = 0.011). In the univariable regression analysis, wider frontal, temporal and parietal sulci span were associated with lower cognitive functions according to MMSE scale.
3. Measurements of both ventricular and cortical brain morphometrics' parameters can be a useful tool for patients' selection for early treatment and preventive strategies.

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CORRESPONDING AUTHOR

Roman Bartiuk
Vinnytsia National Pirogov Memorial Medical University
56 Pirogov st., 21018 Vinnytsia, Ukraine
e-mail: rambrs88@gmail.com

ORCID AND CONTRIBUTIONSHIP

Dmytro Smolko: 0000-0003-3040-3639 A
Roman Bartiuk: 0000-0002-8698-3014 B C
Lesia Zheliba: 0000-0001-9503-6783 D
Yaroslava Marunkevych: 0000-0002-6871-4347 C E
Olga Gordiichuk: 0009-0003-6565-4195 E
Natalia Starynets: 0009-0008-1270-145X E F
Iryna Olkhova: 0000-0002-8855-9428 C D

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

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ORIGINAL ARTICLE

CONTENTS

Dynamics of changes in proteins of the acute phase of inflammation in the postoperative period in patients with disseminated peritonitis

Olga Bilyayeva[1], Ivan Karol[1], Vadim Kryzhevsky[1], Oksana Osadchay[2]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

[2]STATE INSTITUTION «INSTITUTE OF HAEMATOLOGY AND TRANSFUSIOLOGY OF NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE», KYIV, UKRAINE

ABSTRACT

Aim: To determine the effect of the developed complex treatment of patients with peritonitis on the dynamics of humoral factors of nonspecific reactivity in the course of the disease.

Materials and Methods: The study included 124 patients with toxic and terminal stages of peritonitis, who were divided into 3 groups. Group I (main) included 39 patients whose complex treatment included cytochrome C. Group II (main) included 41 patients whose complex treatment included cytochrome C and a solution containing levocarnitine and arginine hydrochloride. The comparison group comprised 44 patients who did not receive the specified drugs. The patients underwent determination of the levels of fibronectin, ceruloplasmin, and procalcitonin in the serum during the course of the disease.

Results: In patients of the I and II main groups, the use of the proposed treatment contributed to the optimization of the production of acute phase proteins: a decrease in procalcitonin production during the study, optimization of ceruloplasmin and fibronectin production, especially in the II main group. In patients of the comparison group, decompensation in the production of humoral inflammatory factors was determined, associated with a significant increase in fibronectin production, a decrease in ceruloplasmin content, and an increase in procalcitonin throughout the entire period.

Conclusions: The use of cytochrome C and a solution containing levocarnitine and arginine hydrochloride in the complex treatment of patients with disseminated peritonitis helps to optimize the production of acute phase proteins, which leads to a decrease in inflammation and the preservation of factors of nonspecific humoral activity at a subcompensated level.

KEY WORDS: peritonitis, acute phase proteins, multiple organ failure, endogenous intoxication, abdominal sepsis

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INTRODUCTION

Peritonitis is a vivid example of inflammation, which has evolved in the course of evolution and is an adaptive and protective reaction of the body that occurs in response to various damaging factors, consists of alternative, vascular and proliferative components and is a good model for studying the general mechanisms of inflammation. As you know, inflammation is one of the oldest forms of the body's defense response to damage factors (mechanical, chemical, thermal, bacterial, ischemic).

Widespread peritonitis and abdominal sepsis is a serious medical and, especially, surgical problem characterized by significant morbidity and mortality. The consequences of peritonitis depend on the local peritoneal and systemic response, as well as on the type, amount of pathogen and duration of its persistence in the abdominal cavity. In a systemic bacterial infection, an intense body response causes excessive production of inflammatory mediators and, as a result, the development of systemic inflammatory response syndrome (SIRS) [1].

Secondary peritonitis is a systemic manifestation of severe peritoneal inflammation secondary to rupture of a hollow organ caused by ischemia or necrosis, previous surgery, or trauma and is associated with an in-hospital mortality rate of approximately 30%, long-term morbidity, and reduced health-related quality of life after 6 months, which leads to increased healthcare costs [2].

Secondary peritonitis is a severe infectious disease of the body characterized by a rapid reaction of the innate immune system and leading to a severe inflammatory process. The initial response is usually accompanied by a depression of innate immunity in various types of sepsis. However, there is a lack of data on the systemic and local innate immune response during peritonitis in humans and its relationship to disease prognosis.

The outcome of secondary peritonitis is influenced by several clinical and bacteriologic features of the disease. For example, two or more microorganisms in the culture of peritoneal fluid, anaerobes, yeasts, or Enterococcus species are associated with a worse prognosis. Also, postoperative peritonitis is associated with a worse outcome and prognosis [3].

Peritonitis is a disease that requires immediate surgical treatment, and abdominal sepsis is a serious problem in the postoperative period. Repeated surgical intervention for abdominal septic foci can be planned or on demand, and improves survival, but relaparotomies are an additional risk factor for patients. Therefore, it is very important to find optimal diagnostic markers for early, noninvasive and reliable diagnosis of intra-abdominal infection and sepsis [4].

Secondary peritonitis is an intra-abdominal infection that requires urgent intervention and rapid antimicrobial therapy to achieve acceptable outcomes. However, a longer duration of antimicrobial therapy is not always associated with improved outcomes and may increase the incidence of resistant strains [5].

Therefore, the determination of humoral factors of nonspecific body reactivity in the treatment of peritonitis can help in the development of a comprehensive treatment of this disease and determine the correct treatment tactics.

AIM

The aim of the study is to determine the effect of the developed complex treatment of patients with peritonitis on the dynamics of humoral factors of nonspecific reactivity in the course of the disease.

MATERIALS AND METHODS

A single-center, single-arm, open-label observational study was conducted at the Brovary Multidisciplinary Clinical Hospital. In 2022, 222 patients with peritonitis were operated on in the surgical department, aged 18 to 93 years. Among them, there were 119 men (53.6%) and 103 women (46.4%).

Patients were examined in accordance with the Declaration of Helsinki of the World Medical Association (Seoul, 2008), orders of the Ministry of Health of Ukraine (№ 281 of November 01, 2000, № 355 of September 25, 2002, No. 356 of 05.22.2009 as amended by the Order of the Ministry of Health of Ukraine No. 574 of 08.05.2009, No. 1118 of 12.21.2012) and with the permission of the Ethics Committee of the Shupyk National University of Health of Ukraine (Protocol No. 8 of 11.7.2022). All patients gave informed consent to participate in the study.

All patients underwent clinical and laboratory examination. The material for this article was based on the medical records of inpatients treated by us, the number of which corresponded to the number of patients in the study (124 records) and the results of laboratory tests. Study inclusion criteria: peritonitis in patients over 18 years of age caused by the following diseases: acute appendicitis, acute cholecystitis, perforated gastric ulcer or duodenal ulcer, tumor perforation, acute destructive pancreatitis, abdominal injuries, small intestinal perforation, abdominal trauma, colonic obstruction of non-tumor origin, small intestinal obstruction. Criteria for non-inclusion in the study: patients with peritonitis of gynecologic genesis.

The study included patients with toxic and terminal stages of peritonitis (124 patients) who were treated in 2022. They were divided into 3 groups. Group I (main) consisted of 39 (31.4%) patients whose complex treatment included cytochrome C, which was administered at a dose of 4 ml (10 mg) twice daily intravenously for 7-10 days. Group II (main) included 41 (33.1%) patients whose complex treatment was supplemented with cytochrome C and a solution containing levocarnitine and arginine hydrochloride, which was administered to patients 100 ml intravenously once daily for 7-10 days. The comparison group consisted of 44 (35.5%) patients in whom the above drugs were not used in the complex treatment of peritonitis. The groups were identical in terms of age and gender.

We studied the activity of humoral inflammatory factors in patients with peritonitis during the course of the disease. Patients underwent the determination of fibronectin, ceruloplasmin and procalcitonin in the blood serum during the course of the disease. The level of fibronectin was determined by enzyme-linked immunosorbent assay, ceruloplasmin - by immunoturbidimetric method, procalcitonin - by immunochemiluminescent assay.

Statistical processing of the study results was performed using the Statistical software EZR v. 1.64 (graphical user interface for R statistical software version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria). Provided that the sample conforms to the law of normal data distribution, statistical hypotheses for equality of means in two dependent or independent samples were evaluated using Student's t or Fisher's F tests at a significance level of 95% ($\alpha = 0.05$). When the indicators did not conform to the law of normal distribution of data, nonparametric statistics methods were used with the use of criteria for testing Wilcoxon-Mann-Whitney (U) and Wilcoxon rank sums (T) statistical hypotheses. In all cases of statistical evaluation, the value of $p<0.05$ was considered significant.

Table 1. Indicators of activity of humoral inflammatory factors in patients with peritonitis of the first main group, M±m, u.s., (n=19)

Analyzed indicators	Units of measurement	Duration of the study, day			Reference values
		1 day	3 days	7 days	
Fibronectin content	μg/ml	105,9±14,7 *p<0,01	120,9±19,2 *p<0,01 **p<0,05	206,1±14,5 *p<0,01 **p<0,01	313,0±22,7
Ceruloplasmin content	mg/dl	66,2±2,7 *p<0,01	57,3±1,9 *p<0,05 **p<0,05	52,4±2,02 *p<0,05 **p<0,05	37,8±4,7
Procalcitonin content	ng/ml	1,41±0,32 *p<0,001	1,12±0,29 *p<0,001 **p<0,05	0,66±0,24 *p<0,001 **p<0,05	0,1±0,8

Notes: * - probability of differences relative to the reference values; ** - probability of differences relative to the initial values.

Table 2. Indicators of activity of humoral inflammatory factors in patients with peritonitis of the second main group, M±m, u.s., (n=21)

Analyzed indicators	Units of measurement	Duration of the study, day			Reference values
		1 day	3 days	7 days	
Fibronectin content	μg/ml	109,5±10,7 *p<0,01	139,2±17,4 *p<0,01 **p<0,05	257,2±12,7 *p<0,05 **p<0,01	313,0±22,7
Ceruloplasmin content	mg/dl	64,7±1,7 *p<0,01	55,7±1,2 *p<0,05 **p<0,05	46,5±1,9 *p<0,05 **p<0,05	37,8±4,7
Procalcitonin content	ng/ml	1,34±0,1 *p<0,001	0,71±0,14 *p<0,001 **p<0,05	0,31±0,37 *p<0,05 **p<0,001	0,1±0,8

Notes: * - probability of differences relative to the reference values; ** - probability of differences relative to the initial values.

RESULTS

When determining the content of fibronectin in the blood serum of patients with peritonitis of the first main group (Table 1), we found a decrease in these indicators relative to the reference values by 2.96 times ($p<0.01$) on the first day of the study.

An increase in the content of ceruloplasmin in the blood plasma was determined. The determined indicators were 1.75 times ($p<0.01$) higher than the reference values. At the same time, an increase in the content of procalcitonin was found in relation to the reference values by 14.1 times ($p<0.001$).

On the 3rd day of the study, we found an increase in fibronectin content by 1.14 times ($p<0.05$) compared to the baseline values, while these values were reduced by 2.59 times ($p<0.01$). A 1.16-fold decrease in ceruloplasmin activity was found relative to the baseline values ($p<0.05$). At the same time, these values exceeded the reference values by 1.52 times ($p<0.05$). There was a tendency to decrease the content of procalcitonin relative to the baseline values by 1.26 times ($p<0.05$), while increasing relative to the reference values by 11.2 times ($p<0.001$).

On day 7 of the study, a tendency to increase the content of fibronectin relative to the baseline values by 1.95 times ($p<0.01$) was determined, but it was reduced relative to the reference values by 1.52 times ($p<0.01$). A decrease in the content of ceruloplasmin and procalcitonin relative to the baseline values was found by 1.26 times ($p<0.05$) and 2.14 times ($p<0.05$), respectively, but they exceeded the reference values by 1.39 times ($p<0.05$) and 6.6 times ($p<0.001$), respectively.

In the study of the content of proteins of the acute phase of inflammation in patients of the second main group (Table 2) on the first day of the study, we found a decrease in the content of fibronectin relative to the reference values by 2.86 times ($p<0.01$). An increase in the content of ceruloplasmin relative to the reference values by 1.71 times ($p<0.05$) and procalcitonin by 13.40 times ($p<0.001$) was determined.

On day 3 of the study, we found a 1.27-fold increase in fibronectin content compared to baseline values ($p<0.05$), while these values were 2.25-fold lower than the reference values ($p<0.01$). A decrease in the concentration of ceruloplasmin relative to the baseline values was determined by 1.16 times ($p<0.05$), while an increase relative to the reference values was 1.47 times ($p<0.05$). A decrease in the content of procalcitonin in the blood plasma relative to the baseline values was

Table 3. Indicators of activity of humoral inflammatory factors in patients with peritonitis of the comparison group, M±m, u.s., (n=17)

Analyzed indicators	Units of measurement	Duration of the study, day			Reference values
		1 day	3 days	7 days	
Fibronectin content	μg/ml	108,5±7,7 *p<0,01	257,7±11,2 *p<0,05 **p<0,01	423,1±16,9 *p<0,05 **p<0,001	313,0±22,7
Ceruloplasmin content	mg/dl	61,4±1,5 *p<0,05	40,1±2,1 *p<0,05 **p<0,05	22,1±1,02 *p<0,05 **p<0,01	37,8±4,7
Procalcitonin content	ng/ml	1,26±0,21 *p<0,001	1,16±0,24 *p<0,001 **p<0,05	1,04±0,31 *p<0,001 **p<0,05	0,1±0,8

Notes: * - probability of differences relative to the reference values; ** - probability of differences relative to the initial values.

found by 1.89 times (p<0.05), but they exceeded the reference values by 7.1 times (p<0.001).

On day 7 of the study, we determined an increase in the content of fibronectin in the blood plasma of patients of the second group compared to the baseline values by 2.35 times (p<0.01), while decreasing relative to the reference values by 1.22 times (p<0.05). An increase in the content of ceruloplasmin relative to the reference data by 1.23 times (p<0.05) was found, while they were reduced relative to the baseline by 1.39 times (p<0.05). At the same time, a decrease in the content of procalcitonin was determined relative to the baseline values by 4.32 times (p<0.001), but they exceeded the reference values by 3.1 times (p<0.05).

In the study of the content of proteins of the acute phase of inflammation in patients of the comparison group (Table 3), we found an increase in fibronectin values relative to baseline values on days 3 and 7 of the study by 2.38 times (p<0.01) and 3.90 times (p<0.001), respectively.

A tendency to decrease ceruloplasmin on days 3 and 7 of the study relative to the baseline values by 1.53 times (p<0.05) and 2.78 times (p<0.01), respectively, was determined. An increase in the content of procalcitonin relative to the reference values was found throughout the study.

Thus, as a result of our studies, we found that in patients with peritonitis, changes in the content of proteins of acute phase of inflammation are determined throughout the study.

DISCUSSION

Fibronectin is a high molecular weight glycoprotein involved in many processes, including cell adhesion, proliferation, embryonic development, and matrix remodeling [6]. Fibronectin significantly accelerates healing and reduces areas of inflammation, and is a significant component of blood clots [7]. In addition, fibronectin plays an important role in the response to infection, participating in maintaining vascular integrity and wound healing, as well as triggering blood clotting processes [8]. It mediates important interactions between phagocytes throughout the inflammatory process and, by forming a three-component bridge, promotes bacterial colonization of endothelial and epithelial cells. A decrease in plasma fibronectin levels is associated with acute inflammation, as well as recent surgical trauma and disseminated intravascular coagulation. In a study of 159 patients with systemic inflammatory response syndrome (SIRS), the authors found a lower level of fibronectin in patients with the presence of microorganisms in the blood than in patients without it (373 μg/ml and 409 μg/ml, respectively) [9]. In our study, we also noted a decrease in fibronectin levels in patients with peritonitis, which on the first day amounted to 105.9-109.5 μg/ml with reference values of 313.0 μg/ml. Under the influence of treatment in patients of the main groups on day 7, the level of this indicator increased to 206.1-257.2 μg/ml.

Ceruloplasmin is an acute-phase plasma protein produced mainly by hepatocytes and activated monocytes and macrophages [10]. In the acute phase, levels of ceruloplasmin, as an inflammatory factor, increase due to the response to infection and inflammation. The role of ceruloplasmin in the body's immunity may be associated with the elimination of free radicals, oxidation and apoptosis of neutrophils, and the inflammatory process [11]. In addition, the ferroxidase activity of ceruloplasmin inhibits the production of reactive oxygen species mediated by iron ions, and thus ceruloplasmin has a powerful antioxidant activity [12]. Despite the fact that the mechanism of ceruloplasmin in copper and iron metabolism has been thoroughly studied,

some questions remain unresolved. Many authors have asked the following questions: Does ceruloplasmin work primarily as an antioxidant or oxidant during oxidative stress? Does it play an anti-inflammatory role in the inflammatory response? Why do studies of ceruloplasmin using different research methods on similar populations give different or even opposite results? In addition, the stability of the physiological functions of ceruloplasmin has not been determined [11]. Our study revealed elevated levels of ceruloplasmin in patients with peritonitis, indicating its active participation in the inflammatory process. Thus, in patients on the first day of the study, ceruloplasmin levels were in the range of 61.4-66.2 mg/dL (with reference values of 37.8 mg/dL). However, with effective treatment and a good patient response to it, the level of ceruloplasmin already on day 7 approached the reference values and amounted to 46.5-52.4 mg/dl in the main groups.

Procalcitonin, a member of the calcitonin superfamily, can be an important tool for the diagnosis of sepsis. Procalcitonin concentrations are associated with the severity of multiple organ dysfunction syndrome, which is secondary to systemic inflammation of infectious origin. A multivariate model showed that higher procalcitonin levels were independent risk factors for septic shock (p=0.046). Thus, according to the authors, the procalcitonin level in patients with septic shock was 54.48±62.14 μg/dL, while in patients without it, it was 0.57±1.31 μg/dL [13]. Procalcitonin helps to increase the concentration of intracellular calcium ions, which facilitate the body's response, decreasing the phagocytic activity of neutrophils in a dose-dependent manner, causes an increase in pro-inflammatory cytokines, aggravates the dysfunction of neutrophils, lymphocytes and macrophages, acts as a powerful trigger of the inflammatory cascade, increases body temperature and motor activity, affects energy homeostasis, cardiovascular stability. These effects can affect the course of the disease in patients with sepsis and are associated with morbidity and mortality. According to the authors, procalcitonin levels in patients with sepsis who survived were 24.90 ± 53.61 μg/dL, while in deceased patients they were at the level of 59.22 ± 99.87 μg/dL [14]. Our study showed the connection of the procalcitonin level with the presence of peritonitis and its dependence on the effectiveness of treatment, as we found significantly elevated levels of procalcitonin in patients with peritonitis on day 1 of the disease. The values were 1.26-1.41 ng/mL (normal range: 0.1 ng/mL). At the end of the study, these values in patients of the main groups were close to the reference values.

In patients of the first and second main groups, the use of the proposed treatment helps to optimize the production of acute phase proteins. These trends were manifested in a decrease in procalcitonin production during the study, which leads to a decrease in inflammation and the preservation of factors of nonspecific humoral activity at a subcompensated level. The optimization of ceruloplasmin and fibronectin production, especially in the second main group, was also determined, which leads to a decrease in oxidative activity and preservation of the antioxidant activity of peripheral blood in the conditions of a generalized infectious process, reduces the risk of complications associated with increased blood coagulation potential, helps maintain vascular integrity and wound healing.

At the same time, patients in the comparison group showed decompensation in the production of humoral inflammatory factors associated with a significant increase in fibronectin production and a decrease in ceruloplasmin content, which may indicate a risk of thrombosis and activation of processes associated with oxidative stress. An increase in procalcitonin throughout the period indicates a significant activity of the inflammatory response and the risk of developing multiple organ failure.

CONCLUSIONS

Determination of the dynamics of the levels of proteins of the acute phase of inflammation, such as fibronectin, ceruloplasmin and procalcitonin in the blood serum, in the course of complex treatment of patients with peritonitis is of great practical and theoretical importance, is an objective indicator of the quality of treatment and allows you to choose the right treatment tactics.

The use of cytochrome C and a solution containing levocarnitine and arginine hydrochloride in the complex treatment of patients with disseminated peritonitis helps to optimize the production of acute phase proteins, which leads to a decrease in inflammation and preservation of factors of nonspecific humoral activity at a subcompensated level. Thus, in patients of the first main group on day 7 of the study, the level of fibronectin was 206.1±14.5 μg/ml, ceruloplasmin - 52.4±2.02 mg/dl, procalcitonin - 0.66±0.24 ng/ml. In patients of the main group II on day 7 of the study, the level of fibronectin was 257.2±12.7 μg/mL, ceruloplasmin - 46.5±1.9 mg/dL, procalcitonin - 0.31±0.37 ng/mL. Whereas in the patients of the comparison group on day 7 of the study, the level of fibronectin was 423.1±16.9 μg/ml, ceruloplasmin - 22.1±1.02 mg/dl, procalcitonin - 1.04±0.31 ng/ml, which indicated decompensation in the production of proteins of the acute phase of inflammation.

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CONFLICT OF INTEREST

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CORRESPONDING AUTHOR

Ivan Karol
Shupyk National Healthcare University of Ukraine
9 Dorohozhytska st, 04112 Kyiv, Ukraine
e-mail: drkarol@ukr.net

ORCID AND CONTRIBUTIONSHIP

Olga Bilyayeva: 0000-0003-2862-0423 A F
Ivan Karol: 0000-0003-3684-0127 B D
Vadim Kryzhevsky: 0000-0002-8538-2583 E
Oksana Osadchay: 0000-0001-5883-425X C

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

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ORIGINAL ARTICLE

CONTENTS

Results of microbiological study of dental biofilm in generalized periodontitis against the background of different body reactivity

Yuriy Yu. Yarov[1], Irina M. Tkachenko[2], Petro M. Skripnikov[2], Olena V. Hurzhii[2], Ruslan V. Kozak[2]
[1]DONETSK NATIONAL MEDICAL UNIVERSITY, KRAMATORSK, UKRAINE
[2]POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Aim: To study the spectrum, frequency ofisolation and level of colonization of dental biofilm with microorganisms in generalized periodontitis against the background of different body reactivity.

Materials and Methods: 216 people with the diagnosis of generalized periodontitis. Depending on the state of reactivity of the organism, the patients were divided into 3 groups: with normo-, hyper- and with hyporeaction. The patients underwent patch surgery. After the surgery, dental biofilm was taken. Microbiological studies included the isolation and species identification of dental biofilm microorganisms, the results of quantitative studies of microflora: the level of colonization was expressed in colony-forming units per 1 ml (CFU/ml); the frequency of microorganisms isolation was expressed in absolute numbers. Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0.

Results: The studies have shown that in different states of the body's reactivityin patients with generalized periodontitis after flap surgery, different quantitative and qualitative composition of the microflora of the dental biofilm is determined. In case of normal body reactivity, there are predominantly aerobic-anaerobic associations with a wide range and quantitative predominance of aerobic microflora; in case of impaired (hyper- and hypo-) reactivity, there are predominantly aerobic-anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes.

Conclusions: The identified differences in the quantitative and qualitative composition of the microflora of the dental biofilm indicate the key role of the body's reactivity in the studied processes.

KEY WORDS: periodontitis, reactivity of the organism, microflora, dental biofilm

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INTRODUCTION

Among the topical problems of modern dentistry, generalized periodontitis is one of the leading ones [1-3]. The key factor in the development of generalized periodontitis is the influence of conditionally pathogenic and specific periodontopathogenic microflora of the dental biofilm, as it initiates the development of inflammation and immunopathological reactions in the periodontium [4-6]. Microorganisms of the dental biofilm are combined into microbial associations, which ensures their coexistence with each other [7]. At the same time, they can even enhance the pathogenic effect of each other (synergism of action) and inhibit the growth of competing microbial species [8,9]. Periodontal pathogens are highly aggressive towards periodontal tissues due to their ability to adhere to the gingival epithelium and invade deep into the tissues (up to the cementum of the tooth root), due to the secretion of destruction enzymes [10,11]. Under the influence of prolonged microbial persistence, the pathological process in periodontal tissues acquires signs of chronic inflammation, which ultimately leads to irreversible destruction of the periodontium and alveolar bone [12,13]. At the same time, complex multicomponent processes of inflammation and destruction occur simultaneously and continuously in periodontal tissues [14]. A complex of microcirculatory, hematological, immunological and connective tissue reactions to damage develops [15-17]. Microcirculatory disorders initiate tissue hypoxia, activation of free radical oxidation, disorganization of biological membranes with the release of physiologically active proinflammatory substances (prostaglandins, cytokines). The outcome of the disease is largely determined by the compensatory abilities of the protective mechanisms of the periodontium and the body as a whole, which are determined by the body's reactivity [18-20].

AIM

The aim of this study was to investigate the spectrum (genus and species of microorganisms), frequency of isolation and level of colonization of dental biofilm with microorganisms after flap surgery in patients with generalized periodontitis of II, III severity degrees against the background of normal, hyper- and hyporeactivity of the body.

MATERIALS AND METHODS

We examined 216 patients (82 men and 134 women) aged 45 to 55 years with a diagnosis of generalized periodontitis II, III severity, chronic course. The diagnosis was made on the basis of clinical examination, radiography, and periodontal samples in accordance with the International Classification of Diseases ICD-10. Depending on the state of the body's reactivity, patients were divided into three groups: the first - normoreaction (132 patients, 61%); the second - hyperreaction (46 patients, 21%); the third - hyporeaction (38 patients, 18%). Patients were divided into groups depending on the state of the body's reactivity based on the identified clinical and laboratory differences. All patients underwent flap surgery as indicated. After surgery (on the 1st day), dental biofilm was taken. The dental biofilm was collected using sterile curettes from the cervical region of the vestibular surfaces of the upper teeth (canines, premolars and first molars), followed by rapid application to a standard sterile swab of the Sarstedt transport system (Germany), which allows to increase the time of transportation to the bacteriological laboratory up to 72 hours.

Microbiological studies included the isolation and species identification of dental biofilm microorganisms using aerobic and anaerobic cultivation techniques by inoculating clinical material from a transport swab onto special nutrient media. Cultivation of the material on the nutrient media was carried out in a thermostat at a temperature of 37 degrees C for 3-5 days. Identification of the isolated pure cultures was carried out by morphological, cultural and biochemical characteristics, as well as by using bioMerieux identification test strips: ARI Staph., ARI 20 Strep., ARI 20 E, ARI 20 A. The results of the quantitative study of microflora: the level of colonization was expressed in colony-forming units per 1 ml (CFU/ml); the frequency of isolation of microorganisms was expressed in absolute numbers.

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

RESULTS

The results of the analysis of the quantitative and qualitative composition of the dental biofilm microflora in patients with generalized periodontitis and normal body reactivity are presented in table (Table 1).

As can be seen from the table, the most common aerobic facultative gram-positive bacteria of the genus *Streptococcus* and *Staphylococcus*, respectively, were found in 77.2 and 87.1% of cases. Their spectrum is represented by the following species: *Str. mitis, Str. mutans, Str. intermedius, S. haemolyticus, S. hominis. In isolated cases, S. auricularis, S. warneri, S. aureus and S. schleiferi* were found. It should be noted that such species of *Streptococcus* and *Staphylococcus* as *Str. pyogenes, Str. faecium, Str.equinus, S. cohnii, S. simulans, S. lentus* were not detected in patients with AP of this group. When assessing the level of colonization of the dental biofilm with aerobic cocci, it was found that the number of CFU of bacteria in 1 ml of clinical material on average, is 10^6. Facultative gram-negative bacilli *Escherichia coli* and *Enterobacter* were found, respectively, in 77.7 and 8.3% of cases. At the same time, such representatives of this group of bacilli as *Klebsiella*, *Citrobacter* and *Capnocytophaga* were not detected in the dental biofilm. In 24.2% of cases, bacteria of the genus *Peptostreptococcus* (*species P. anaerobius, P. prevotii*) and the genus *Porphyromonas* (*species P. gingivalis*) were isolated, in 15.9% of cases - bacteria of the genus *Prevotella* (*species P. oralis*). In addition, gram-negative anaerobic bacteria of the genus *Fusobacterium (species F. Nucleatum* and *F. necrophorum)* were detected in 65.27% of patients. The average number of CFU of these anaerobes in 1 ml of clinical material was $10 - 10^{57}$. Anaerobic bacteria of the general *Actinomyces* and *Bacteroidas*, as well as aerobic bacteria of the genus *Pseudomonas* were not detected. Thus, the analysis of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis with normal body reactivity on the 1st day after surgery indicates the presence of predominantly aerobic-anaerobic associations with a wide range and quantitative predominance of aerobic microflora.

The results of the analysis of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis against the background of hyperreactivity of the body indicate a number of differences from such parameters in patients with normal reaction. As can be seen from the table, aerobic facultative gram-positive bacteria of the genus *Streptococcus* and *Staphylococcus* were found in the dental biofilm of persons of this group in 56.5% of cases, which is significantly less frequent compared to the first group. The spectrum of detected aerobes was significantly narrowed in patients of the second group compared to the normal reaction. Thus, the number of streptococci species decreased by 3 species: *Str. mitis*, *Str. mutans*, *Str. intermedius*. The assessment of the level of dental biofilm colonization with streptococci showed that it was commensurate with the values in the first group - the number of CFU of bacteria in 1 ml of clinical material was on average 10^6 . At the same time, the level of staphylococci colonization, on average, reached 10^5 , which is 10 times

Table 1. The frequency of microorganisms isolation and the level of dental biofilm colonization in normal, hyper- and hyporeactivity of the body on the 1st day after surgical treatment (abs. / CFU / ml)

Genus and species of isolated microorganisms		norm-reaction (n = 132)	hyper- reaction (n = 23)	hypo- reaction (n = 19)
Aerobic and facultative coccigenus Staphylococcus	*Staphylococcus*	115 / 10 -10^{48}	13 / 10 -10^{28}	10 / 10 -10^{28}
	S. haemolyticus	21/10^{8}	6 / 10 -10^{24}	7 / 10 -10^{24}
	S. auricularis	18/10^{5}	4/10^{6}	3/10^{6}
	S. capitis	12/10^{7}	4/10^{4}	3/10^{4}
	S. hominis	10/10^{4}	3/10^{4}	3/10^{4}
	S. warneri	9/10^{4}	3/10^{4}	2/10^{4}
	S. aureus	-	4/10^{5}	2/10^{5}
	S. lentus	-	-	-
	S. schleiferi	-	3/10^{8}	2/10^{8}
	S. cohnii	-	-	-
	S. simulans	-	-	-
Aerobic and facultative cocci genus Streptococcus	*Streptococcusspp.*	102 / 10 -10^{58}	13 / 10 -10^{57}	9 / 10 -10^{57}
	Str.sanguis	-	7 / 10 -10^{57}	6 / 10 -10^{57}
	Str.mitis	42 / 10 -10^{58}	-	-
	Str.mutans	30/10^{7}	-	-
	Str. pyogenes	-	-	-
	Str. Faecium	-	5/10^{5}	4/10^{7}
	Str.intermedius	22 / 10 -10^{58}	-	-
	Str.equinus	-	5/10^{8}	4/10^{8}

less than in patients with a normal reaction. In addition to aerobic cocci, the appearance of aerobic gram-negative bacilli of the genus *Pseudomonas* was noted in the dental biofilm. It should be noted that the spectrum of facultative gram-negative bacilli has also expanded, the frequency of detection and the level of colonization have increased. Thus, representatives of the genera *Klebsiella* and *Capnocytophaga* appeared. In addition, the number of cases of isolation of *Escherichia coli* and *Enterobacter* from the bacillus has increased. The expansion of the spectrum, the increase in the frequency of isolation and the level of colonization of the dental biofilm with gram-positive and gram-negative anaerobes is noteworthy. Thus, anaerobic bacteria of the genera *Actinomyces* and *Bacteroidas* were detected; the frequency of isolation of *Peptostreptococcus*, *Porphyromonas*, *Prevotella* and *Fusobacterium* increased. At the same time, the level of colonization of the dental biofilm with *Peptostreptococcus, Porphyromonas and Fusobacterium* increased 5-10 times. Thus, the analysis of the quantitative and qualitative composition of the dental biofilm microflora in patients with generalized periodontitis against the background of increased body reactivity indicates the predominance of aerobic- anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes.

The results of the analysis of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis against the background of hyporeactivity of the body on the 1st day after surgery indicate their similarity to those in hyperreactivity of the body and a number of differences from such parameters in normal body reactivity. As can be seen from the table, aerobic facultative gram-positive bacteria of the genus *Streptococcus* and *Staphylococcus* were found in the dental biofilm, respectively, in 47.4 and 52.6% of cases, which is comparable to the values in hyperreactivity and much less frequent compared to the values in normoreactivity. At the same time, the spectrum of detected aerobes was similar to that in the second group and significantly narrowed compared to that in the first group. Assessment of the level of colonization of the dental biofilm with streptococci showed that it was commensurate with the values in the normal body reactivity - the number of CFU of bacteria in 1 ml of clinical material, on average, was 10^{6}. At the same time, the level of staphylococci colonization, on average, reached 10^{5}, which is 10 times less than that of patients in the first group. In addition to aerobic cocci, the appearance of aerobic gram-negative bacilli of the genus *Pseudomonas* was noted in the dental biofilm. It should be noted that the composition of the dental biofilm also expanded the spectrum, increased the frequency of detection and the level of colonization of facultative gram-negative bacilli compared to that of the normoreactivity of the organism. Thus, representatives of the genera *Klebsiella* and *Capnocytophaga* appeared. In addition, the number of cases of isolation of

Escherichia coli and *Enterobacter* bacilli has increased. As well as against the background of increased reactivity of the organism, the expansion of the spectrum, increase in the frequency of isolation and the level of colonization of dental biofilm with gram-positive and gram-negative anaerobes is noteworthy. Thus, anaerobic bacteria of the genera *Actinomyces* and *Bacteroidas* were found in the content of the dental biofilm; the frequency of isolation of *Peptostreptococcus*, *Porphyromonas*, *Prevotella* and *Fusobacterium* increased. At the same time, the level of colonization of the dental biofilm by *Peptostreptococcus*, *Porphyromonas* and *Fusobacterium* increased 5-10 times. Thus, the analysis of the quantitative and qualitative composition of the dental biofilm microflora in patients with generalized periodontitis against the background of reduced body reactivity on the 1st day after surgery, as well as in case of increased body reactivity, indicates the presence of predominantly aerobic- anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes.

DISCUSSION

Generalized periodontitis has all phases of inflammation: alteration, exudation, and proliferation. Wound healing is also an inflammatory reaction characterized by a cascade of neuro-regulatory, biochemical, neuro-trophic, immunological and functional disorders, microcirculatory and metabolic disorders [2]. The healing of the postoperative wound after flap surgery in patients with generalized periodontitis II, III degrees of severity also represents an inflammatory reaction with the appropriate ratio of the phases of necrotic and reparative processes. It is known that the intensity and duration of the inflammatory reaction during the healing of a myocardial infarction is determined by the form of the initial reactivity of the body and determines the complicated and uncomplicated consequences [21]. In the conducted study, we showed that in different states of the body's reactivity in patients with generalized periodontitis after flap surgery, different quantitative and qualitative composition of the microflora of the dental biofilm is determined. In case of normoreactivity of the organism, there are predominantly aerobic-anaerobic associations with a wide range and quantitative predominance of aerobic microflora; in case of impaired (hyper- and hypo-) reactivity, there are predominantly aerobic-anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes. The revealed differences in the quantitative and qualitative composition of the dental biofilm microflora indicate the key role of the organism's reactivity in the studied processes.

CONCLUSIONS

In the dental biofilm of patients with generalized periodontitis with impaired (hyper- and hypo-) reactivity of the body after surgery, an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes was found compared to that in the normal reaction. We consider the correction of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis with impaired body reactivity with bringing it to the values of normal reactivity as a condition for optimizing postoperative wound healing and further stabilization of the process in periodontal tissues. Therefore, we consider it promising to develop methods of targeted drug correction that will bring microbiological parameters to those corresponding to the normoreactivity of the body.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Yuriy Yu. Yarov
Donetsk National Medical University
39 Mashonobudivnikov Boulevard, 84404 Kramatorsk, Ukraine
e-mail: kaf.intern.stomat@ukr.net

ORCID AND CONTRIBUTIONSHIP

Yuriy Yu. Yarov: 0000-0002-2867-9866 A B C D E F
Irina M. Tkachenko: 0000-0001-8243-8644 A D
Petro P. Skripnikov: 0000-0002-4473-2284 A C
Olena V. Hurzhi: 0000-0003-1711-2335 C E
Ruslan V. Kozak: 0000-0003-1711-2335 A F

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

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ORIGINAL ARTICLE

CONTENTS

Xylate effect on the renal toxicological function with diabetes mellitus complicated by endogenous intoxication syndrome of purulent-septic genesis

Viktor Konovchuk, Andrii Andrushchak, Sergij Kushnir, Denys Stoliar, Petro Moroz
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

Aim: To study xylate effect on the renal toxin-excreting function with diabetes mellitus (DM) complicated by endogenous intoxication syndrome of purulent-septic genesis.

Materials and Methods: The effect of infusions with sorbilact or Ringer's solution in the regimen 3 ml/kg/hour during 3 hours on toxin-excreting function of the kidneys in patients with type 2 DM complicated by EIS is studied.

Results: In the period of the research, xylate increased cleaning blood plasma (extracellular water space) from the total toxicity by 6,0±1,9 ml/min (230±72,3%, Δ p<0,05). Ringer's solution infusion in the fragment of intensive care of the same group of patients (n=53) was determined by increase of clearance of toxic substances by 4,3±1,2 ml/min (165±46,0%, Δ p<0,05). At the same time, xylate infusion decreased the total blood plasma toxicity by 22±4,6 IU/ml (14±2,9%, Δ p<0,05), and Ringer's solution – by 12±3,9 IU/ml (7±2,2%, Δ p Ringer's solution) in patients with Type 2 DM complicated by endogenous intoxication syndrome of purulent-septic genesis. At the same time, xylate infusion reduced the total plasma toxicity by 22±4.6 IU/ml (14±2.9%, Δ p<0.05), Ringer's solution - by 12±3.9 IU/ml (7±2.2%, Δ p<0.05).

Conclusions: Infusion therapy solutions (xylate, Ringer's solution) within the study regimen (3 ml/kg/h for three hours) activate the renal excreting function and reduce the level of toxemia (xylate > Ringer's solution) in patients with Type 2 DM complicated by endogenous intoxication syndrome of purulent-septic genesis. At the same time, xylate infusion reduced the total plasma toxicity by 22±4.6 IU/ml (14±2.9%, Δ p<0.05), Ringer's solution - by 12±3.9 IU/ml (7±2.2%, Δ p<0.05).

KEY WORDS: type 2 DM, renal toxin-excreting function, xylate, endogenous intoxication syndrome of purulent-septic genesis

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INTRODUCTION

Infusion therapy is a basic one in the intensive therapy of biological integrity disorders of a patient organism [1]. It is prioritized due to the content of ingredients essential to maintain homeostasis and correction of its quality. Today, association of diabetes mellitus with endogenous intoxication syndrome (DMEIS) including that of purulent-septic genesis is rather spread nosology [2]. It requires a comprehensive treatment, careful monitoring, individualization of intensive therapy measures considering numerous complications of diabetes mellitus and endogenous intoxication syndrome factors focused on the formation of multiple organ damage [3]. Therefore, the issues concerning the choice of infusion therapy medicines in order to rehabilitate the condition of target organs with diabetes mellitus associated with metabolic disorders initiated by the concomitant syndrome of endogenous intoxication deserve certain attention [4]. One of the answers to this question is within the range of the action of an appropriate drug used for the infusion therapy through the prism of the renal toxin-excreting function. Xylate can be such a drug, since its capabilities correspond to the requirements for the correction of this pathology. Its main component xylitol is a natural intermediate product of carbohydrate metabolism in people and animals. Its pharmacokinetics is not associated with special hormonal or enzymatic transport mechanisms. Its degree of assimilation does not depend on the age of the patient. According to the mechanism of action, it binds the pentose phosphate cycle with uronic acid metabolism. The final product of oxidation is carbon dioxide eliminated by the lungs. Compared to glucose, xylitol is more effective source of energy conversion, and its metabolism does not depend on insulin. It possesses lipotropic and antiketogenic activity, stimulates glycogen synthesis in the liver and insulin secretion, improves microcirculation, normalizes water-salt metabolism and acid-alkaline state, and possesses detoxification action with burn disease, protracted purulent processes and infectious diseases [5].

AIM

The aim of the work was to study xylate effect on the renal toxin-excreting function with diabetes mellitus complicated by endogenous intoxication syndrome of purulent-septic genesis.

MATERIALS AND METHODS

The research is open randomized, prospective and controlled. The patients suffering from type 2 DM complicated by endogenous intoxication syndrome of purulent-septic genesis were examined. Inclusion criteria include type 2 DM, age of patients – 42-65 years, the determined period of the disease – 5-12 years. The previous history: dietary correction, specific regimen, hypoglycemic agents, insulin (9%); actual blood glucose – 8-16 mmol/L, glycosylated hemoglobin > 7%, transient microalbuminuria (30-300 mg/day); different clinical signs of angiopathy and neuropathy are registered without considerable disturbances of the afflicted organs and systems. The course of type 2 DM of these patients was complicated by acute surgical infection after surgical rehabilitation of foci of various localization caused by association of aerobic gram-positive and gram-negative flora. The disease was associated with by endogenous intoxication syndrome of purulent-septic genesis of 20-50 points on the intoxication cellular humoral index scale [6].

All the patients (n=53) received appropriate surgical rehabilitation and a comprehensive standard intensive therapy. Sugar level was corrected in the hospital by insulin in case glucose level > 10 mmol/L. In case of inclusion into the research, the intensive therapy of patients was supplied by infusions of Ringer's and xylate solutions. The indicators characterizing the renal toxin-excreting function were studied in the interval of the test Ringer's solution infusion and 20-24 hours later – xylate. The group characteristics were determined: I-IV groups – type 2 DM including A: indicators of patients before Ringer's solution (I group) or xylate infusion (III group); B – after Ringer's solution (II group) or xylate infusion (IV group). The regimen of solution infusions was 3 ml/kg/hours during 3 hours. After increasing the extracellular space volume with solutions, patients were examined within a 4-hour interval considering the time of infusion load. The parameters of values examined in biological medium including total blood plasma (urine) toxicity [6], concentration of average mass molecules in the blood plasma or urine [7], cellular-humoral intoxication index [6], procalcitonin [8] are presented in the Table. The data obtained were statistically processed by means of Student T-criterion for the dependent (Δ) and independent samples (statistical package Excell, trial version) [9].

RESULTS AND DISCUSSION

The two periods were determined in the course of infusion therapy for the patients with DMEIS (n=53): the first one deals with the examination of Ringer's solution effect (3 ml/kg/hours during 3 hours) on the renal toxin-excreting function (I-II groups); the second one – after 20-24 hours of xylate effect (III-IV group) under the same conditions of the study (Table 1)..

Unicellular receptor systems are sensitive indicators of the total toxicity of the blood plasma (Рт) [10]. As a result of modification [6], it becomes informative as a criterion of activity of the renal toxin-excreting function, balance between toxin formation and toxemia. It enables to evaluate detoxification effect of the solutions in the intensive care (IC) program (Table 1).

Administration of xylate was associated with an increased secretion of toxic substances by 766±118,9 IU/ml (177±27,8%, Δ $p<0,05$). Analysis of this process constituents, that is, concentration characteristics of the total toxicity of urine ingredients – before xylate load and after it (Table 1) with diuresis volume of these patients A – 1,23±0,03 ml/min, B – 3,58±0,08 ml/min, $p<0,05$), is indicative of a prevailing xylate effect on water excretion kidney function and its importance in the formation of the total blood plasma toxicity, since the total urine toxicity decreased.

In the control studies, where Ringer's solution was used (Table 1) excretion of toxic substances increased as well (by 646±117 IU/ml, or by 151±32,6%,Δ $p<0,05$). The volume of excretion of toxic substances by kidney nephrons depends on their filtration ability (filtration fraction), reabsorption, and may be secretion. In its turn, the value of excreted fraction (Table 1) demonstrates a part of toxic substances which is eliminated with urine from the filtration fraction. Xylate infusion in particular, increased excretory fraction by 4,9±0,8%, (Δ $p<0,05$), Ringer's solution by 2,2±0,6%, (Δ $p<0,05$). An increased excretion of toxic substances by the kidney nephrons under conditions of a standardized glomerular filtration rate (based on 100 ml/min of glomerular filtration rate) were 623±144,0 IU/ml, (Δ $p<0,05$) and 531±130,8 IU/ml, Δ $p<0,05$) respectively. Meanwhile, the parameters of excretion of toxic substances are more indicative of the kidney functional state. In order to get an idea of the effectiveness of the used solutions in cleaning the extracellular water space from toxic substances, in particular, in the case of endogenous intoxication syndrome caused by the combination of diabetes mellitus with purulent-septic complications, the clearance characteristics were applied, reflecting the stability of homeostasis (Table 1). Xylate administered during the examination period increased blood plasma (extracellular water sector) clearance from total toxicity

Table 1. Effect of xylate and Ringer's solution on toxin-excreting function of the kidneys with type 2 DM complicated by endogenous intoxication syndrome of purulent-septic genesis

Indices	Diabetes mellitus (n=53) (M±m)			
	I group (A)	II group (B)	III group (A)	IV group (B)
P_T, IU//ml	167±3,6	156±3,7**	161±3,7	140±3,8* **
U_T IU/ml	349±4,2	330±4,3**	352±4,4	335±4,5**
U_TV, IU/min	426±4,8	1070±7,3**	433±4,9	1199±7,5* **
U_TV/GFR*100, IU/min	361±4,1	892±9,3**	360±4,0	983±9,9* **
EF_T, %	2,2±0,04	4,4±0,05**	2,1±0,05	7,0±0,06* **
C_T, ml/min	2,6±0,05	6,9±0,11**	2,6±0,06	8,6±0,12* **
P_{MMM}, IU/ml	0,71±0,019	0,64±0,021**	0,69±0,02	0,57±0,020* **
U_{MMM}, IU/ml	11,7±0,21	9,4±0,19**	11,2±0,22	9,3±0,23**
$U_{MMM}V$, IU/min	14,3±0,32	30,5±0,69**	13,8±0,30	33,3±0,71* **
$U_{MMM}V$/GFR*100, IU/min	12,1±0,16	25,4±0,52**	11,5±0,25	27,3±0,58* **
EF_{MMM}, %	17,2±0,20	39,7±0,59**	17,6±0,21	47,9±0,67* **
C_{MMM}, ml/min	20,1±0,57	47,7±1,22**	20,2±0,59	58,4±1,27* **
CHII, score	47±0,94	42±0,91**	45±0,92	38±0,93* **
BPP, ng/ml	4,3±0,09	4,0±0,11**	4,0±0,09*	3,6±0,13* **

Note: I group - IV group – DM; A – patients before Ringer's solution infusion (I group) or xylate (III group); B – patients before Ringer's solution infusion (II group) or xylate (IV group);
*/p≤0,05 – reliability of indices between I group and III, II and IV groups,
**/p≤0,05 – reliability of indices between I group and II; III group and IV group.
P_T – total toxicity of blood plasma;
U_T – urine toxicity;
U_TV – excretion of toxic substances;
U_TV/GFR*100 – excretion of toxic substances per 100 ml GFR;
EF_T – excreted fraction of toxic substances;
C_T – clearance of toxic substances;
P_{MMM} – MMM concentration in blood plasma;
U_{MMM}– MMM concentration in urine;
$U_{MMM}V$ – MMM excretion;
$U_{MMM}V$ /GFR*100 – excretion of toxic substances per 100 ml GFR;
EF_{MMM} – excreted fraction of MMM;
C_{MMM} – MMM clearance;
CHII– cellular humoral intoxication index;
BPP– blood plasma procalcitonin.

by 6.0±1.9 ml/min (230±72,3%, Δ p<0,05). Infusion of Ringer's solution in IT fragment in the same patients (n=53) was marked by increased clearance by 4,3±1,2 ml/min (165±46,0%,Δ p<0,05). Thus, the effectiveness of the administered solutions demonstrated itself in the form of an integrative indicator change – the total toxicity of the blood plasma (Table 1). Under observation conditions, xylate infusion reduced the total toxicity of blood plasma by 22±4.6 IU/ml (14±2,9%, Δ p<0,05), and Ringer's solution – by 12±3,9 IU/ml (7±2,2%,Δ p<0,05).

Among the numerous factors of endogenous intoxication, which are included in the total toxicity of the blood plasma, medium mass molecules (MMM) are distinguished. They possess high biological activity, in particular, neurotoxic, cardiotoxic, hepatotoxic, nephrotoxic activity. Their organotropic action is stipulated by the property to inhibit biosynthesis of protein and enzymes; separate oxidation and phosphorylation processes; perform toxic action on erythropoiesis etc. [11]. MMMs (300-5000 D) are the components of endotoxicosis with endogenous intoxication syndrome. They are freely filtered by the kidney glomeruli and create a high concentration in the ultrafiltrate of the nephron proximal portion. There, the major amount of MMMs is metabolized by the peptidase system of the nephrothelium to amino acids that are reabsorbed. A part of MMMs is excreted by the kidneys. Therefore, activity of MMMs elimination will depend on the

glomerular filtration rate, proximal metabolism, and activity of reabsorption-secretory processes. Considering the fact that MMMs are one of the known fractions causing EIS development, participation of the kidneys in elimination of toxemia components is an important criterion to assess the course of EIS and determine the tactics of infusion therapy.

One of the integrative parameters characterizing the balance between the intensity of MMMs formation and their elimination from the body is MMM concentration index in the blood plasma (Table 1). After xylate infusion, MMM concentration in the blood plasma decreased 0,12±0,032 IU/ml (17±4,4%, Δ p<0,05). In the group of comparison (Ringer's solution infusion) the dynamics of changes was similar (decrease by 0,07±0,024 IU/ml or 10±3,7%, Δ p<0,05).

Among the mechanisms of cleaning the blood plasma from MMMs certain processes in the kidneys are considered. First of all, it is determination of MMM filtration ability. The latter is indicative of the fact that xylate and Ringer's solution in the nephrons of patients suffering from DMEIS does not increase MMMs flow from the glomeruli to the proximal portion of nephrons, since the administered solutions with this mode of introduction did not practically change glomerular filtration rate (I group A – 118±1,7 ml/min; II group B – 120±1,9 ml/min; III group A – 120±1,8 ml/min; IV group B – 122±1,9 ml/min) and produced hemodilution effect.

However, due to decrease in proximal metabolism and reabsorption, the excreted fraction of MMMs (showing how much MMMs from the filtration fraction is eliminated in the urine after reabsorption) after administration of xylate increased by 2.7 times, and after Ringer's solution infusion – by 2.3 times (Table 1). At the same time, nephron activity concerning MMMs excretion under conditions of the standardized glomerular filtration rate (Table 1), after xylate administration increased by 137±37,6%, (Δ p<0,05), and after Ringer's solution infusion – by 110±35,5%, (Δ p<0,05). Eventually, MMMs excretion by the kidneys increased by 177±27,8%,Δ p<0,05 and 151±32,6%, respectively (Δ p<0,05). This index is a constituent to calculate the effect of solutions on the clearance processes of the extracellular space from MMMs by the kidneys in patients with DMEIS. The findings are presented in the Table 1. It should be noted, that at this stage of the research in the regimen of infusion therapy (3 ml/kg/hours during 3 hours), xylate promoted increase of the volume of extracellular fluid clearance by 38,2±8,1 ml/min (189±40,1%,Δ p<0,05); and Ringer's solution – by 27,6±7,9 ml/min respectively(137±42,0%, Δ p<0,05). The reaction and practical value of the cellular humoral index of intoxication and concentration of procalcitonin in the blood plasma after administration of xylate of Ringer's solution are indicative of the level of their importance with the given course of toxemia in patients with DMEIS (Table 1).

CONCLUSIONS

Infusion therapy solutions (xylate, Ringer's solution) within the study regimen (3 ml/kg/h for three hours) activate the renal excreting function and reduce the level of toxemia (xylate > Ringer's solution) in patients with Type 2 DM complicated by endogenous intoxication syndrome of purulent-septic genesis. At the same time, xylate infusion reduced the total plasma toxicity by 22±4.6 IU/ml (14±2.9%, Δ p<0.05), Ringer's solution - by 12±3.9 IU/ml (7±2.2%, Δ p<0.05).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Denys Stoliar
Bukovinian State Medical University
2 Theater square, 58002 Chernivtsi, Ukraine
e-mail: stolyar@bsmu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Viktor Konovchuk: 0000-0003-2231-4185 A C D
Andrii Andrushchak: 0000-0002-0320-2383 B C D
Sergij Kushnir: 0000-0002-3784-0324 B
Denys Stoliar: 0000-0002-0345-9349 C E F
Petro Moroz: 0000-0002-7131-8863 C

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

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ORIGINAL ARTICLE

CONTENTS

Comprehensive morphological study of free radical processes in chronic chorioamnionitis on the background of iron deficiency anemia in pregnancy

Vitalii V. Ilika, Oleksandra V. Garvasiuk, Oleksandra I. Dogolich, Serhii S. Malaiko, Iryna V. Batih
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

Aim: To establish the features of free radical processes in the endotheliocytes of the chorionic plate of the placenta in chronic chorioamnionitis against the background of iron deficiency anemia of pregnant women using both chemiluminescent and histochemical methods of research.

Materials and Methods: 82 placentas from parturients at 37 – 40 weeks of gestation were studied. Including, for comparison, the placenta during physiological pregnancy and the observation of iron deficiency anemia of pregnant women without inflammation of the placenta. The number of observations in specific study groups is given in the tables. To achieve the objective and solve the tasks set in this study, there were carried out the following histochemical, chemiluminescent, morphometric and statistical methods of material processing.

Results: In case of chorionamnionitis against the background of anemia in pregnancy, the R/B ratio (R/B - ratio between amino- (blue) and carboxyl (red) groups of proteins)) in the method with bromophenol blue according to Mikel Calvo was 1.56±0.021, indicators of chemiluminescence of nitroperoxides were 133±4.5, relative optical density units of histochemical staining using the method according to A. Yasuma and T. Ichikawa was - 0.224±0.0015

Conclusions: With chronic chorioamnionitis, the intensity of the glow of nitroperoxides, the average indicators of the R/B ratio, and the optical density of histochemical staining for free amino groups of proteins are increased compared to placentas of physiological pregnancy and anemia of pregnant women. Comorbid i anemia of pregnant women causes increasing of the intensity of the glow of nitroperoxides, the average values of the R/B ratio, and the optical density of histochemical staining for free amino groups of proteins comparing to placentas with inflammation without anemia. The key factor in the formation of morphological features of chronic chorioamnionitis with comorbid anemia is the intensification of free radical processes, which is reflected by the increase in the concentration of nitroperoxides in the center of inflammation, with the subsequent intensification of the processes of oxidative modification of proteins, which is followed by the increasing activity of the processes of limited proteolysis.

KEY WORDS: chorioamnionitis, oxidative modification of proteins, limited proteolysis, free radical processes, iron deficiency anemia in pregnancy

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INTRODUCTION

In case of both inflammation of the placenta and iron deficiency anemia in pregnancy (IDAP), one or other signs of placenta insufficiency are almost always registered, which can have adverse consequences for the fetus [1-3].

In addition, there is a strong cross-relationship between hypoxia and inflammation. Inflammation creates hypoxic conditions due to increased metabolic activity; hypoxia at the site of inflammation can cause vascular damage, blood coagulation and blood flow blockage, and subsequently tissue necrosis [4]. In this regard, there are studies in the modern literature aimed at studying the influence of the inflammatory process on the clinical course of IDAP, however, no information about morphological manifestations in the placenta with comorbidity of these conditions was found in the studied scientific literature.

It is also known that free radical processes are activated both during inflammation and during IDAP. This can potentially cause their mutual aggravation and the development of more severe pathology of the placenta. An imbalance between the occurrence of reactive oxygen species and the ability of antioxidants to inhibit oxidative damage leads to damage to macromolecules, and therefore to impaired placental cell function, defects in the exchange of nutrients between the mother and the fetus [5-6]. The cases described in the national and international literature do not cover all pathomorphological changes in the placenta caused by free radicals both in inflammation and in iron deficiency anemia. No data was found when combining these states either. For this purpose, this paper is aimed at a comprehensive study of the state of free radical processes in individual structures of the placenta using modern histochemical and fluorescent methods.

AIM

To establish the features of free radical processes in the endotheliocytes of the chorionic plate of the placenta in chronic chorioamnionitis against the background of iron deficiency anemia of pregnant women using both chemiluminescent and histochemical methods of research.

MATERIALS AND METHODS

82 placentas from parturients at 37 – 40 weeks of gestation were studied. Including, for comparison, the placenta during physiological pregnancy and the observation of iron deficiency anemia of pregnant women without inflammation of the placenta. The number of observations in specific study groups is given in the table (Table 1).

To achieve the objective and solve the tasks set in this study, there were carried out the following histochemical, chemiluminescent, morphometric and statistical methods of material processing:

1. Histochemical methods. Pieces of placenta were fixed for 24 to 48 hours in neutral Lilly-buffered 10% formalin solution, followed by ethanol dehydration and paraffin pouring according to standard procedures. On serial histological sections of 5 μm thick there were performed 3 methods:
   - with bromophenol blue according to the Mikel Calvo method to determine the degree of oxidative modification of proteins (OMB). Performed in the modification of I.S. Davydenko [7-9], which included 4 stages: 1) histochemical stage (staining with bromophenol blue according to Mikel Calvo); 2) optical stage (application of microscopic optics); 3) photographic stage (application of digital photo documentation); 4) computer microspectrometric stage (color analysis on digital microphotographs).
   - by the method of A. Yasuma and T. Ichikawa (technique using ninhydrinoschiffian reaction to free amino groups of proteins);
   - with bromophenol blue according to Bonheg (method for total protein morphometric).
2. Chemiluminescence method (determination of nitroperoxides in the studied structures). To enhance luminescence, frozen sections of the placenta were treated for 5 minutes (37 °C) with a solution of luminol mixed with stabilizers according to the prescription. Immediately, without washing off the solution, the preparations were studied in a LUMAM-R8 microscope with immediate photofixation of the glow by a digital camera under standard conditions for 5 minutes, when the glow remains stable (the same).
3. Morphometric research methods. In order to objectify quantitative studies, computer morphometry of objects in histological and histochemical preparations was performed. First, digital copies of the optical image of the sections of the microscopic preparations were first obtained using a Delta Optical Evolution 100 microscope (planachromatic lenses) and an Olympus SP-550UZ digital camera using different microscope lenses depending on the purposes of the analysis (eyepiece 10x, lens 10x – 20 x – 40 x).

The obtained digital copies of the images were analyzed using a licensed copy of the computer program Image J (1.48, W. Rasband, National Institutes of Health, USA) according to the intended purpose. Thus, to measure the degree of oxidative modification of proteins, spectral characteristics were determined using computer microspectrophotometry (decomposition of the color of the object into two components - red and blue, with a quantitative assessment of each part of the spectrum) in the RGB color evaluation system according to the algorithm [8]. Using computer microdensitometry, optical density was determined on photocopies of histological images obtained by the method of Bonheg (for total protein) and A. Yasuma and T. Ichikawa (for evaluation of limited proteolysis). The optical density of histochemical staining was measured in relative units of optical density in the range from 0 - no staining, absolute transparency, to 1 - maximum staining, absolute opacity, based on logarithmic transformations of the brightness value in gradations from 0 to 255.

On the obtained images of samples of the chemiluminescent method of research, the intensity of luminescence was determined by computer evaluation on a scale of 256 gradations - from 0 (the indicator of the absence of luminescence) to 255 (the maximum intensity of luminescence), which made it possible to carry out quantitative measurements of luminescence (units of luminescence).

4. Statistical methods of material processing. Statistical processing of digital data was carried out on a personal computer depending on the specific task of a certain fragment of the study using a free-to-use licensed copy of the computer program PAlaeontological STatistics (PAST) v3.15 [10].

The arithmetic mean and its error (M±m) were calculated. Differences in mean values were tested using an unpaired Student's t-test after positive testing of the sample for normality of distribution in it by the Shapiro-Wilk test. The critical value of the level of significance (p) was accepted at $p \leq 0.05$, which could indicate the influence of iron deficiency anemia in pregnancy on the course of free radical processes during inflammation of the placenta. If a value of $p > 0.05$ was obtained, the difference between the values was considered unreliable.

Table 1. The results of histochemical research methods for "acidic" and "basic" proteins, as well as for total protein with bromophenol blue according to Mikel Calvo, for free amino groups of proteins according to the method of A. Yasuma and T. Ichikawa, chemiluminescence study of nitroperoxides (M ± m)

Research groups	Physiological pregnancy (n=20)	Observation of iron deficiency anemia of pregnant women without inflammation of placenta (n=21)	Observation of placenta inflammation during pregnancy without anemia (n=20)	Observation of placenta inflammation in iron-deficiency anemia of pregnant women (n=21)
Histochemical technique for "acidic" and "basic" proteins with bromophenol blue according to Mikel Calvo (R/B ratio)	0,81±0,018	0,98±0,023 p<0,001	1,38±0,016 p_1<0,001 p_2<0,001	1,56±0,021 p_3<0,001 p_4<0,001
Chemiluminescent glow of nitroperoxides (unit of lum.)	18±4,8	35±4,7 p=**0,01**	112±4,2 p_1<0,001 p_2<0,001	133±4,5 p_3=**0,001** p_4<0,001
Histochemical technique according to the method of A. Yasuma and T. Ichikawa on free amino groups of proteins (relative optical density units)	0,164±0,0019	0,168±0,0018 p>0,05	0,205±0,0021 p_1<0,001 p_2<0,001	0,224±0,0015 p_3<0,001 p_4<0,001
Histochemical technique for total protein with bromophenol blue according to Bonheg (relative optical density units)	0,234±0,0108	0,230±0,0091 p>0,05	0,233±0,00104 p_1>0,05 p_2>0,05	0,230±0,0092 p_1>0,05 p_2>0,05

Note. P_1 is the probability of the difference between the two means between physiological pregnancy and the studied group; P_2 is the probability of the difference of two means between the group of placentas with IDAP and the studied group; P_3 is the probability of the difference between the two means between inflammation and inflammation in combination with IDAP. P_4 is the probability of the difference between the two means between inflammation in combined with IDAP and IDAP without inflammation.

Correlation analysis was performed using the Pearson (r) method.

All studies were performed in compliance with the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (March 18, 1986) "Ethical Principles for Medical Research Involving Human Subjects", approved by the Declaration of Helsinki (1964 - 2013), ICH GCP (1996), EEC Directives #609 (dated 11/24/1986), Orders of the Ministry of Health of Ukraine #690 dated 09/23/2009, #944 dated 12/14/2009, #616 dated 08/03/2012. The Commission on Bioethics of the Bukovinian State Medical University of Ukraine found no violations (Minutes #4 of December 19, 2019). All the procedures and experiments of this study respect the ethical standards of the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law. Informed consent was obtained from all the patients included in the study.

RESULTS

In order to study the processes of oxidative modification of proteins in the endotheliocytes of the chorionic plate of the placenta, the R/B ratio was determined by the microspectrometric method on digital copies of the images after the histochemical technique for "acidic" and "basic" proteins with bromophenol blue according to Mikel Calvo. The R/B ratio served as an indicator of the ratio between amino and carboxyl groups in proteins of a specific localization, that is, it was a measure of OMP.

Upon visual assessment, it is established that endotheliocytes are quite clearly stained, well recognized due to their characteristic localization, that is, they are suitable for quantitative research. The cytoplasm of endotheliocytes is mostly stained diffusely, although sometimes individual, darker granular formations can be observed. Endotheliocyte nuclei are not visualized - the image of the cell nucleus merges with the cytoplasm. This distinguishes the endotheliocyte from many other types of cells. Therefore, the ratio of "acidic" and "basic" proteins in an endotheliocyte cannot be histochemically studied separately for the cytoplasm and the cell nucleus, that is, only together, in general.

The average quantitative indicators of the R/B ratio in the studied groups are presented in the Table 1.

Since OMP in cells occurs under the action of free radicals, in order to determine nitroperoxides, as the largest long-lived free radicals capable of overcoming relatively significant diffusion distances and therefore able to cause real pathology in the depth of the tissue, and not only on the surfaces [11-12], a chemiluminescent research method (fig. 3) was conducted with luminol, followed by measurement of luminous intensity

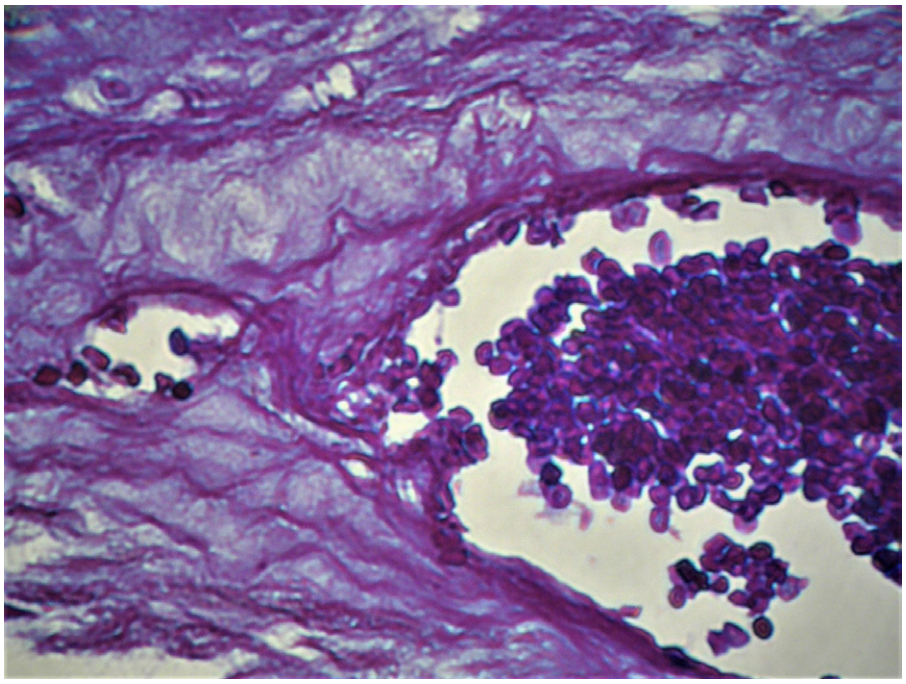

Fig. 1. Observation of chronic chorioamnionitis against the background of iron deficiency anemia in pregnancy. Endothelium of the chorionic plate. Staining for amino and carboxyl groups of proteins. Histochemical technique according to Mikel Calvo. Magnification: Lens 40x, eyepiece 10x.

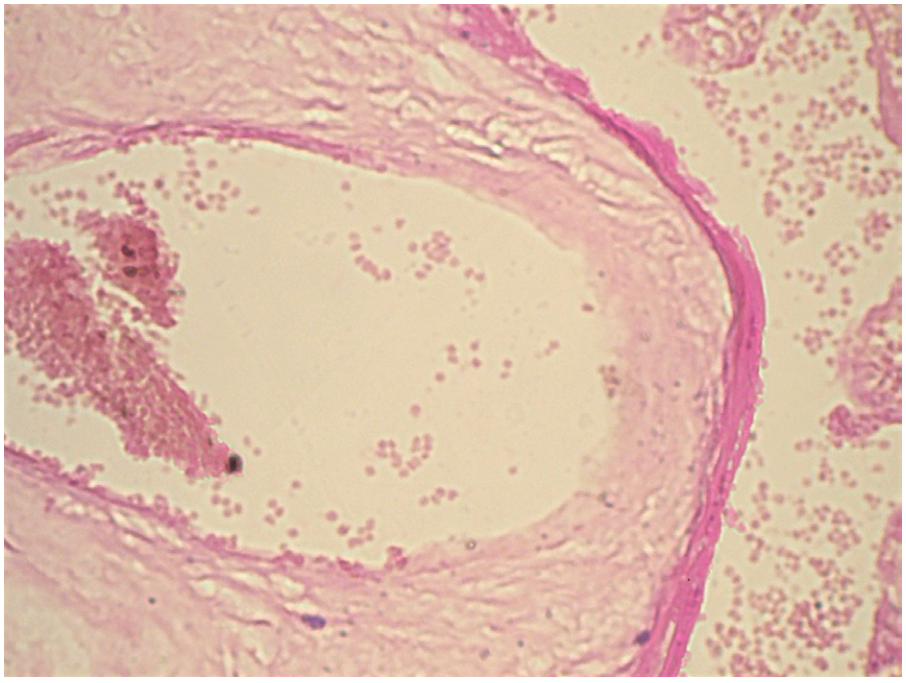

Fig. 2. Observation of chronic chorioamnionitis against the background of iron deficiency anemia in pregnancy. Endothelium of the chorionic plate. Specific staining for free amino groups of proteins. Ninhydrin-Schiffov reaction according to the method of A. Yasuma and T. Ichikawa. Magnification: Lens 40x, eyepiece 10x.

(relative units of luminosity). Photomicrographs show that chemiluminescence after luminol treatment was irregular among endotheliocytes, but differences in luminescence depending on the zone of the chorionic plate of the placenta were not detected. Outside the endothelium of blood vessels, there are faintly visible foci of luminescence, which may indicate the ability of nitroperoxides to overcome comparatively larger diffusion distances far beyond the boundary of the foci of their origin.

The average quantitative indicators, according to the results of the studies in the endotheliocytes of the blood vessels of the chorionic plate, are shown in the Table (Table 1).

It is known that during many pathological processes, as a rule, the level of free oxygen radicals increases, which, by oxidizing the amino groups of proteins, change the properties of these macromolecules [12]. Intensification of the processes of oxidative modification of proteins may be accompanied by enhanced processes of limited proteolysis.

To study these processes, a histochemical technique was performed according to the method of A. Yasuma and T. Ichikawa, which allows using the ninhydrin-Schiff

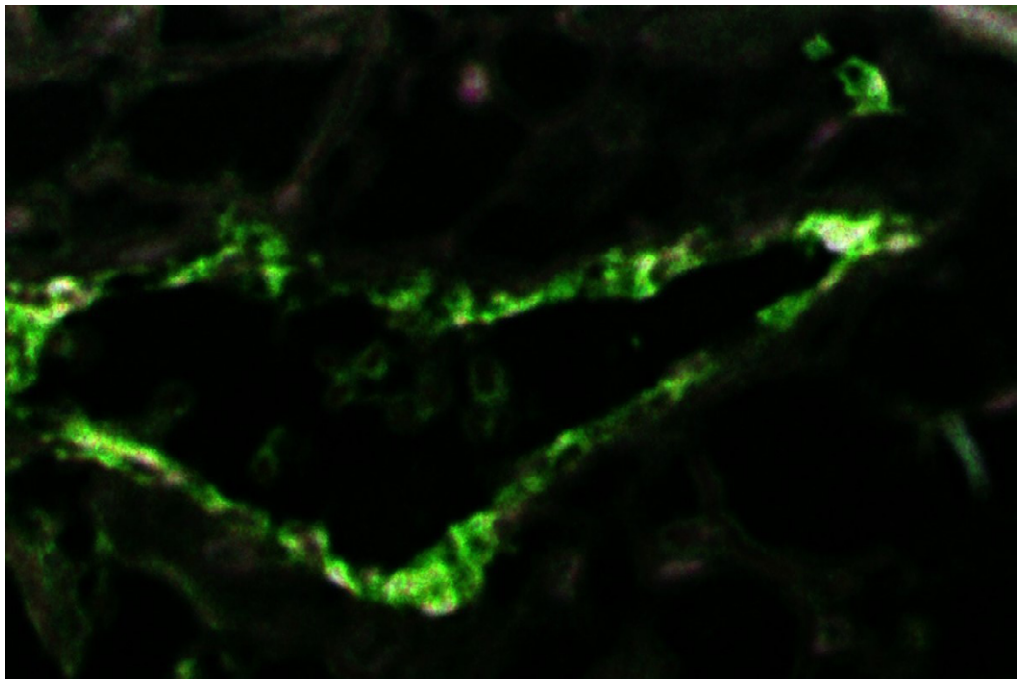

Fig. 3. Observation of chronic chorioamnionitis against the background of iron deficiency anemia in pregnancy. Endothelium of the chorionic plate. Chemiluminescence of nitroperoxides. Chemiluminescence study with luminol Magnification: Lens 40x, eyepiece 10x.

reaction to specifically stain free NH2 (amino groups) of proteins. In order to be able to interpret the obtained data regarding limited proteolysis, quantitative determination of total protein was additionally carried out by histochemical method with bromophenol blue according to Bonheg. The results of the study are shown in the table (Tabl. 1).

Analyzing the obtained results of the study, highlighted in the Table, we can note that in case of IDAP, the R/B ratio and the intensity of the nitroperoxide glow increase with a statistically probable discrepancy ($p<0.05$), in the absence of changes in the quantitative indicators of the optical density of histochemical staining for free amino groups of proteins according to the method of A. Yasuma and T. Ichikawa, and for total protein with bromophenol blue according to Bonheg ($p>0.05$).

In case of chronic chorioamnionitis, the R/B ratio, the intensity of the nitroperoxide glow, and the relative units of the optical density of histochemical staining for free amino groups of proteins significantly increase on average compared to the placentas of physiological pregnancy and iron-deficiency anemia of pregnant women without placenta inflammation ($p<0.001$). However, the indicators of the optical density of histochemical staining for total protein with bromophenol blue according to Bonheg remain without statistically significant differences compared to the groups of comparisons ($p>0.05$).

In the observations of chronic chorioamnionitis against the background of iron deficiency anemia in pregnancy, a sharp increase in quantitative indicators was observed when using all research methods ($p</=0.001$), with the exception of an increase in total protein indicators in the histochemical method for total protein with bromophenol blue according to Bonheg ($p>0.05$).

Below there are microphotographs of placenta sections stained with bromophenol blue according to Mikel Calvo for "acidic" and "basic" proteins. (fig. 1), according to the method of A. Yasuma and T. Ichikawa on free amino groups of proteins (fig. 2), as well as photocopies of preparations obtained by chemiluminescence of nitroperoxides with luminol (fig. 3).

DISCUSSION

Morphological assessment of oxidative stress in placental tissue structures should be comprehensive and include many pathohistological research methods. First of all, considerable attention should be paid to the determination of radicals, in particular nitroperoxides, which, due to their longer existence, are able to overcome greater diffusion distances and therefore are able to cause real pathology in the middle of the tissue, not only on its surface [13] and are subject to quantitative studies.

In our study, it is determined that in the placentas from parturient women who were diagnosed with iron deficiency anemia, an increase in the level of nitroperoxides was observed in the endotheliocytes of the blood vessels of the chorionic plate of the placenta among all the studied structures (35±4.7 units of lum. at physiological pregnancy indicators - 18 ±4.8 units of lum., $p=0.01$). The results close to ours were obtained by A.V. Hoshovska et al. [13] in the study of nitroperoxides in the cytoplasm of the trophoblast of chorionic villi in case of IDAP.

At the same time, based on the following research results, the relationship between free radicals and the inflammatory process should not be underestimated, because free radicals actively participate in the development of the inflammatory reaction, starting from the alteration phase and ending with reparation processes [14]. Thus, studying nitroperoxides in the endotheliocytes of the blood vessels of the chorionic plate of the placenta in chronic chorioamnionitis (112±4.2 units of blood, p<0.001, with normal values - 18±4.8 units of blood, p<0.001;) we noticed an increase in brightness intensity compared to the norm. The same trend was observed by I. S. Davydenko et al. [15], who used the chemiluminescent technique with luminol to determine nitroperoxides in the syncytiotrophoblast cytoplasm of the chorionic villi of the placenta in purulent chorioamnionitis. After it was carried out, the average quantitative indicators were statistically higher than the indicators of physiological pregnancy.

With regard to comorbid IDAP during inflammation, we received data on the intensification of the chemiluminescent glow of nitroperoxides (133±4.5 unit of lum., p=0.001).

Let's try to find a logical explanation of the obtained quantitative indicators of the chemiluminescent glow of nitroperoxides during inflammation.

This is primarily due to the fact that the endothelium is one of the tissues most sensitive to the effects of hypoxia [16] and is the first to be activated during inflammation. When endothelial cells and smooth muscle cells are stimulated, there is an excess of radicals, in particular superoxide anion radical (O2), which easily reacts with free nitric oxide (NO), forming a highly reactive and toxic compound - peroxynitrite (ONO^2). This can be one of the main reasons for the development of a vasoconstrictor effect, disruption of micro- and macrocirculation processes, metabolic disorders in tissues and their acceleration, and, ultimately, acceleration of the processes of apoptosis and necrosis in cells [17].

In addition, the sources of reactive radicals can be both intracellular structures (mitochondria, xanthine oxidase), and extracellular ones - neutrophils and macrophages. Lymphocytes and fibroblasts also constantly generate small amounts of superoxide radicals. And since these cellular elements are constantly present in large quantities during inflammation, this confirms the high concentration of radicals in the foci of inflammation [11].

A high concentration of nitroperoxides in the center of inflammation should be considered as a trigger for the intensification of the processes of oxidative modification of proteins, since reactive oxygen species can be considered as the main inducers of OMP [18].

We used the histochemical technique for "acidic" and "basic" proteins with bromophenol blue according to Mikel Calvo. The essence of this research method is that the oxidation of amino groups of proteins leads to a change in the ratio between amino and carboxyl groups in them, and when staining histological sections with bromophenol blue, proteins are colored in different colors. In particular, basic proteins are colored blue, acidic proteins are colored red.

We evaluated colors using computer microspectrophotometry on digital copies of images in the RGB color evaluation system. The final result of the study was the R/B ratio, which is actually a quantitative expression of the ratio between amino and carboxyl groups of proteins and an indicator of the degree of oxidative modification of proteins.

During the histochemical study of placentas with IDAP, there was an increase in the R/B ratio in the endotheliocytes of the chorionic plate of the placenta (p<0.05). The obtained results complement the conclusions of scientists who studied the process of OMP in other structures of the placenta with IDAP [19].

Taking into account the data we obtained earlier about the high level of nitroperoxides during placenta inflammation, the increase in the R/B ratio is most correctly associated with the increase in the intensity of OMP processes. Thus, our results showed that in the group of placentas with chronic ChA, in the endotheliocytes of the chorionic plate of the placenta with a high statistical probability (p<0.001), the processes of OMP increased (1.29±0.019 units of lum.) and even more in inflammation against the background of IDAP.

To confirm cause-and-effect relationships, we conducted a correlation analysis using the Pearson method between observations of inflammation in comorbid iron-deficiency anemia of pregnant women. Thus, strong correlations were established between the intensity of the nitroperoxide glow and OMP processes according to the R/B ratio, where r=0.793 (p<0.05), which can confirm the induction of OMP processes by free radicals when these states are combined.

Increased formation of free radicals under the influence of hypoxia caused by iron-deficiency anemia of pregnant women with a natural increase in the processes of oxidative modification of proteins should be accompanied by an increase in free amino groups of proteins. This was confirmed by the histochemical method of research according to the method of A. Yasuma and T. Ichikawa in combination with microdensitometry, both in chronic inflammation of the chorionic plate (0.205±0.0021, p<0.001) and against the background of IDAP (0.224±0.0015, p<0.001). We obtained similar results when studying the processes of limited proteolysis in the fibrinoid of both the chorionic and basal plates of patients with inflammation against the background of anemia of pregnancy [20].

The peculiarity of this technique is that it detects only free groups of proteins. Therefore, this technique shows the number of unhidden (free) amino groups of proteins rather than the total amount of protein. If there is a loss of proteins of a higher degree of structural organization (denaturation of proteins), the hidden amino groups of proteins become free - available for oxidation by ninhydrin. Therefore, in order to be able to interpret the obtained data on limited proteolysis, there was conducted a quantitative determination of total protein (using the method with bromophenol blue according to Bonheg) [20]. The results of the study are shown in the Table (Table 1). At the same time, we did not find any signs of changes in the concentration of total protein in the endotheliocytes of the chorionic plate of the placenta ($p>0.05$), which indicates the fact of sufficient regeneration of proteins in the cells. As a result, this causes the protein to lose its ability to perform its functions and protein denaturation occurs.

In order to find a relationship between the intensity of the brightness of nitroperoxides and the processes of limited proteolysis, the correlation coefficient was determined using the Pearson method. Thus, according to the indicators of the optical density of histochemical staining, $r=0.704$ ($p<0.05$), and the correlation of limited proteolysis with OMP processes with statistical probability ($p<0.05$) was $r=0.783$.

The obtained results are an addition to the hypothesis about the intensification of free radical processes during inflammation of the placenta, which manifests itself with a certain regularity: an increase in the intensity of the glow of nitroperoxides, which indicates a high concentration of them in the center of inflammation, an increase in the R/B ratio, which is a reflection of OMP processes, and an increase in optical density histochemical staining for free amino groups of proteins as an indicator of the activity of limited proteolysis processes.

This gives reason to believe that the key factor in the formation of morphological features of inflammation of the placenta is the intensification of free radical processes, and iron deficiency additionally significantly modifies these processes, which in turn enhances proteolysis [20].

CONCLUSIONS

1. With iron-deficiency anemia in pregnancy, the intensity of the glow of nitroperoxides and the average indicators of the R/B ratio increase compared to placentas of physiological pregnancy.
2. With chronic chorioamnionitis, the intensity of the glow of nitroperoxides, the average indicators of the R/B ratio and the optical density of histochemical staining for free amino groups of proteins increase compared to the placentas of physiological pregnancy and iron-deficiency anemia of pregnant women without inflammation.
3. Comorbid iron-deficiency anemia in pregnant women increases the intensity of the glow of nitroperoxides, the average indicators of the R/B ratio and the optical density of histochemical staining for free amino groups of proteins compared to placentas with inflammation without anemia.
4. The key factor in the formation of morphological features of chronic chorioamnionitis with comorbid iron deficiency anemia is the intensification of free radical processes, which is reflected by the increase in the concentration of nitroperoxides in the center of inflammation, with the subsequent intensification of the processes of oxidative modification of proteins, which is followed by the increasing activity of the processes of limited proteolysis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Vitalii V. Ilika
Bukovinian State Medical University
2 Theatrical Sq., 58000 Chernivtsi, Ukraine
e-mail: vitaliy.ilika@bsmu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Vitalii V. Ilika: 0000-0002-3714-9883 A C D F
Oleksandra V. Garvasiuk: 0000-0002-1936-2015 B E
Oleksandra I. Dogolich: 0000-0002-5309-2602 C E
Serhii S. Malaiko: 0000-0001-9306-7850 E
Iryna V. Batih: 0000-0002-8498-921X B

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

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ORIGINAL ARTICLE

CONTENTS

Histological and immunohistochemical features of the placenta associated with COVID-19: a systematic review and meta-analysis

Gulsym S. Manasova[1], Yana A. Stasy[1], Vyacheslav V. Kaminsky[2], Igor Z. Gladchuk[1], Ekaterina A. Nitochko[1]
[1]ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE
[2]P. I. SHUPIK NATIONAL UNIVERSITY OF HEALTH, KYIV, UKRAINE

ABSTRACT

Aim: To make a systematic review and meta-analysis of published data on the study of histological and immunohistochemical features of the placenta in women who had acute coronavirus infection associated with SARS-CoV-2 ("Covid" placentas) during pregnancy.

Materials and Methods: The search for literature data is based on the PRISMA methodology); the MEDLINE database (PubMed®) was searched using Medical Subject Headings terms from January 2020 to July 2023. The project was registered in the Open Sience Frame (Project Identifier: DOI 10.17605/OSF.IO/GDR3S, Registration DOI: https://doi.org/10.17605/OSF.IO/H2KPU). Preference was given to studies in which the description of placentas met the requirements of the Amsterdam Placental Workshop Group Consensus Statement.

Results: A total of 31 studies were included; the number of participants whose morphological and histological description of the placentas could be subjected to meta-analysis was 2401, respectively, in the group with a "Covid" history and 1910 – conditionally healthy pregnant women. Pathological changes in the placental complex were not detected in 42±19.62% of pregnant women with a history of Covid. Immunohistochemical examination of placentas preferably focuses on the detection of SARS-CoV-2 spike protein or ACE2. According to currently available studies, in the placentas of women who have had COVID-19 during pregnancy, there are no pathognomic histological patterns specific to this infection and direct damage to the placenta is rarely observed. Histological patterns in "covid" placentas are isolated, most often a combination of lesions in both the maternal and fetal malperfusion.

Conclusions: According to currently available studies, in the placentas of women who have had COVID-19 during pregnancy, there are no pathognomic histological patterns specific to this infection and direct damage to the placenta is rarely observed. The probability of infection of the intrauterine fetus by the transplacental hematogenous route is the lowest compared to other routes, which, in our opinion, is a possible explanation for the high frequency of MVM without subsequent infection of the fetus.

KEY WORDS: Pregnancy, maternal/fetal vascular malperfusion, SARS-CoV-2, morphological features of the placenta

Wiad Lek. 2024;77(7):1434-1455. doi: 10.36740/WLek202407120 DOI

INTRODUCTION

Coronavirus disease (COVID-19) pandemic during 2019-2022. has become one of the most important medical and social problems of the society due to high contagiousness, rapid spread and high mortality rate. Due to the natural physiological immunodeficiency state of the body, pregnant women are among the most vulnerable groups of the population exposed to the adverse effects of infectious agents. This raised concerns in the medical community about the spread of COVID-19 among this cohort of patients [1, 2]. First observations showed asymptomatic or mild clinical course of the disease among pregnant women [3, 4], serious complications in this group were rarely observed. Also, there was no reliable confirmation of infection vertical transmission - from mother to fetus, so that was similar to previous coronavirus diseases (severe acute respiratory syndrome - SARS, Middle East respiratory syndrome - MERS) and other RNA respiratory viruses infections [5, 6].

As the pandemic spread and involved more and more people in the Western Hemisphere, Europe and the Middle East, cases of severe pneumonia and multiple organ failure syndrome began to be reported among pregnant women, with a natural increase for intensive care and mechanical ventilation; cases of maternal mortality began to be reported [7, 8].

Publications also began to appear on the possibility of vertical transmission of SARS-CoV-2-associated coronavirus infection from mother to fetus and morbidity in newborns [9, 10, 11, 12,13]. But there is currently insufficient information on the impact of the SARS-CoV-2 virus on the placenta and newborn to obtain convincing evidence [14-16].

In accordance with the physiology of pregnancy, the placenta plays the role of an important multifunctional mediator in the interaction between the organisms of mother and intrauterine fetus. During gestation, various organs of the fetus gradually take over all the functions of the placenta, including the lungs, liver, intestines, kidneys and endocrine glands, but the main function of the placenta - to ensure optimal metabolism and perfusion in the maternal-fetal bloodstream is retained. Optimal perfusion is ensured by structural remodeling of the uterine arteries and the formation of a large contact area between the placenta and the thin interhemal membrane separating the maternal and fetal circulation. Various maternal conditions leading to morphological and metabolic changes at the maternal-fetal interface can affect placental function [17, 18].

Information about the absence of a significant impact of COVID-19 on the course of pregnancy and perinatal outcomes [19, 20, 21], as well as information about the low probability of vertical transmission of infection and damage to the fetus [22, 23], does not exclude the possibility that the placenta may be infected with a virus and have certain histopathological features.

So, according to Şahin O. et al. (2022), the placenta of women with asymptomatic COVID-19 is characterized by the presence of parietal hypertrophy of arteriolar membranes [24]. In severe cases of SARS-CoV-2, an associated infection in "Covid" placentas, other authors have identified varying degrees of histiocytic intervillositis, perivillous deposition of fibrin and trophoblast necrosis, as well as cases of fetal vascular malperfusion (FVM) [25].

Based on Schwartz DA et al. data (2022), a characteristic feature of SARS-CoV-2-associated placentitis is increased fibrin deposition and necrosis of trophoblast villous tissue, as well as chronic histiocytic intervillositis. The authors note that an average of about 77.7% of the placental tissue is involved in the inflammatory destructive process [26].

In another work, Schwartz DA et al. (2022), when analyzing cases of stillbirth in pregnant women with COVID-19, note that infection and destruction of the placenta can be observed in the absence of proven infection in the fetus. It is assumed that the development of SARS-CoV-2-associated placentitis may be the result of viremia and have both an infectious and immunological basis. It is also emphasized that in all registered cases of stillbirth and neonatal losses with subsequent verification of placentitis, the mothers were not vaccinated [27].

There is evidence of the absence of any SARS-CoV-2-specific histopathological changes in the placenta, despite vascular malperfusion in the maternal and/or fetal bloodstream, inflammatory changes and other patterns. The authors consider direct infection of the placenta by the SARS-CoV-2 virus to be a rare event [28].

Currently, most histological and immunohistochemical studies of "Covid" placentas are devoted to confirming infection of the placenta with the SARS-CoV-2 virus [28, 29, 30, 31, 32] and studying directly in the placenta the expression of angiotensin-converting factor receptors (ACE2), through which one of the main damaging effects of SARS-CoV-2 is realized [33, 34, 35]. It is believed that the vertical transmission of SARS-CoV-2 is critically dependent on the presence of the ACE2 receptor in the placenta [36, 37].

Usually, not all placentas require routine histological examination; indications are justified by concomitant mother's diseases or complications of pregnancy, when this particular study can provide insight into the likely (and unlikely) causes of prenatal and intrapartum problems and prognostic information for the mother and child. Lack of information, relevant studies, lack of follow-up period, different inclusion standards and limitations of systematic reviews make it difficult to accurately interpret the data [14, 38-41].

AIM

The purpose of this work: to make a systematic review and meta-analysis of published data on the study of histological and immunohistochemical features of the placenta in women who had acute coronavirus infection associated with SARS-CoV-2 ("Covid" placentas) during pregnancy.

MATERIALS AND METHODS

The systematic literature review project was registered in Open Science Frame (Project Identifier: DOI 10.17605/OSF.IO/GDR3S, Registration DOI: https://doi.org/10.17605/OSF.IO/H2KPU), inclusion and exclusion criteria for studies are presented in Suppl.1 (OSF Registries | COVID-19 in Pregnancy and morphological and histological features of the placenta: a preliminary review | Files) .

The literature search was based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) methodology [42]; the MEDLINE database (PubMed®) was searched using combinations of the relevant Medical Subject Headings terms (MeSH), key words, and word variants for "COVID-19 and pregnancy," COVID-19 and placenta," "SARS- CoV-2 and the placenta" and "COVID-19 and histology of the placenta", "COVID-19 and immunohistochemistry of the placenta" (Suppl. 2. OSF Registries | COVID-19 in Pregnancy and morphological and histological features of the placenta: a preliminary review | Files). Papers in English were se-

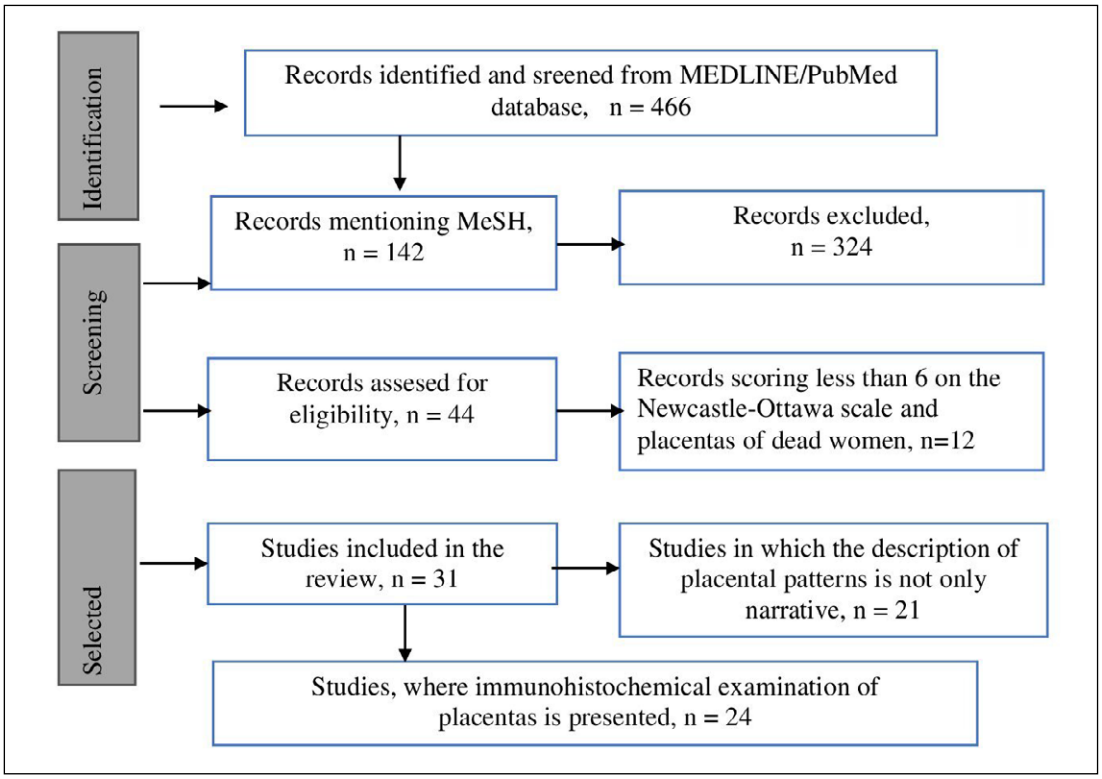

Fig. 1. PRISMA flow chart of database search.

lected for analysis in 3 stages (identification of relevant studies, selection and final selection) based on MeSH/ abstracts; in the case of lack of access to the full text or lack of information on the morphological-histological and immunohistochemical examination of placentas, the works were excluded from further analysis (Fig. 1).

Preference was given to studies that described placentas in accordance with the Amsterdam Placental Workshop Group Consensus Statement (APWGCS, 2016); which contained information not only of a descriptive nature about data such as Maternal vascular malperfusion (MVM), Fetal vascular malperfusion (FVM), Acute and Chronic inflammatory pathology (AIP, CIP) and other criteria [43]. All search results for the period January 2020 - July 2023, taking into account the recommendations of The Joanna Briggs Institute [44], were checked by two independent experts from different Ukrainian institutions and different cities (Fig. 1), the potential significance of the studies and the final selection were agreed upon during the discussion.

Taking into account the requirements for non-randomized studies, a questionnaire was developed for selection and methodological assessment of the quality of published works - the Newcastle-Ottawa Scale (NOS) [45], which included information about the diagnosis of Covid-19 in pregnant women, histological and immunohistochemical examination of placentas as one of the diagnostic methods for this diagnosis, information about the comorbid status of patients, the course of pregnancy, and perinatal outcomes (Table 1)

Studies were required to contain information on confirmation of SARS-CoV-2 infection by PCR (polymerase chain reaction); in the description of placentas, the presence of macroscopic examination, electron microscopy of placenta samples with hematoxylin and eosin staining, and immunohistochemical studies aimed at identifying inflammatory, immune-mediated and other changes in the placenta were taken into account.

STATISTICAL ANALYSIS

The statistical analysis was performed using the online software https://www.socscistatistics.com/tests. After determining the normality of the distribution with the use of the Kolmogorov-Smirnov test, a comparative analysis of the data was carried out. For the descriptive analysis of the study group, categorical variables were summarized as percentages and continuous variables as means or medians with the corresponding standard deviation (SD). Categorical variables were compared between groups using T-test calculator for 2 independent means and the Relative Risk (RR) and Odds Ratio (OR) calculator. The threshold for statistical significance was set at 0.05.

RESULTS

The total number of studies that generally met the stated selection criteria was 30 of the initially identified 466 studies (Table 2).

Table 1. The Newcastle-Ottawa Scale -NOS)

№	Author	Pregnant women with COVID-19 (PCR)	Histological examination of the placenta	IHC examination of the placenta (for ACE 1-2 and other)	Healthy pregnant women (control group)	Comorbid status	pregnancy complications	Perinatal outcomes	Gestational age	IHC examination of the placenta for COVID-19
1.	Adam A.M. [12].	*	*	*	*	*	*	*	*	NP
2.	Bertero L. [49].	*	*	NP	*	*	*	*	*	*
3.	Boyraz B. [67.	*	*	*	*	-	*	-	*	*
4.	C. Tasca C. [22].	*	*	*	*	*	*	*	*	*
5.	Celik E. [13].	*	*	*	NP	*	*	*	*	*
6.	Corbetta-Rastelli C.M. [23].	*	*	NP	NP	*	*	*	*	NP
7.	Damman E. [30].	*	*	*		*	*	*	*	*
8.	Di Girolamo R. [40].	*	*	*	*	NP	*	*	NP	*
9.	Edlow A.G. [36].	*	*	*	*	NP	*	*	NP	*
10.	Facchetti F. [39].	*	*	*	*	NP	NP	*	*	*
11.	Ferraz T. [51].	*	*	NP	*	*	*	*	*	*
12.	Gao L. [29].	*	*	*	NP	*	*	*	*	*
13.	Giordano G. [66].	*	*	*	NP	*	*	*	*	*
14.	Glynn S.M. [58].	*	*	*	*	*	*	*	*	*
15.	Gychka S.G. [15].	*	*	*	*	*	NP	*	*	*
16.	Horn L.C. [67].	*	*	*	NP	*	*	*	*	*
17.	Hsu A.L. [41].	*	*	*	NP	*	*	*	*	*
18.	Husen M.F. [69].	*	*	*	NP	*	*	*	*	*
19.	Jaiswal N. [71].	*	*	NP	*	NP	*	*	*	*
20.	Kosič N. [16].	*	*	*	NP	*	*	*	*	*
21.	Levitan D. [28].	*	*	*	*	*	*	*	*	*
22.	Linehan L. [31].	*	*	*	NP	*	*	*	*	*
23.	Marton T. [72].	*	*	*	NP	NP	NP	*	*	*
24.	Rakheja D. [46].	*	*	*	NP	NP	NP	NP	*	*
25.	Rebutini PZ. [59].	*	*	*	*	*	*	*	*	*
26.	Şahin O. [24]	*	*	NP	*	*	*	*	*	NP
27.	Sharps M.C. [60].	*	*	*	*	*	*	*	*	*
28.	Smithgall M.C. [32].	*	*	*	*	*	*	*	*	*
29.	Surekha M.V. [47].	*	*	NP	*	*	*	*	*	NP
30.	Taglauera E.S. [35].	*	*	*	*	*	*	*	*	*
31.	Watkins J.C [25].	*	*	*	NP	*	*	*	*	*

Note: Authors - only the first author is listed; (-) – absent; ACE - angiotensin converting enzyme; NP - not presented; IHC – Immunohistochemical; PCR - polymerase chain reaction.

Of the 30 studies, 9 described the placentas in a narrative manner and, thus, 21 studies were selected for meta-analysis in which the pathological patterns of "Covid" placentas were not only descriptive in nature, but could also be assessed statistically and allowed for a meta-analysis . It should be noted that not all of these works described placentas fully complied with the requirements of APWGCS; information on the number of studies with this or that characteristic of histological patterns is presented in Fig. 2.

Taking into account the peculiarities of COVID-19 pathogenesis, the prevalence of thrombotic compli-

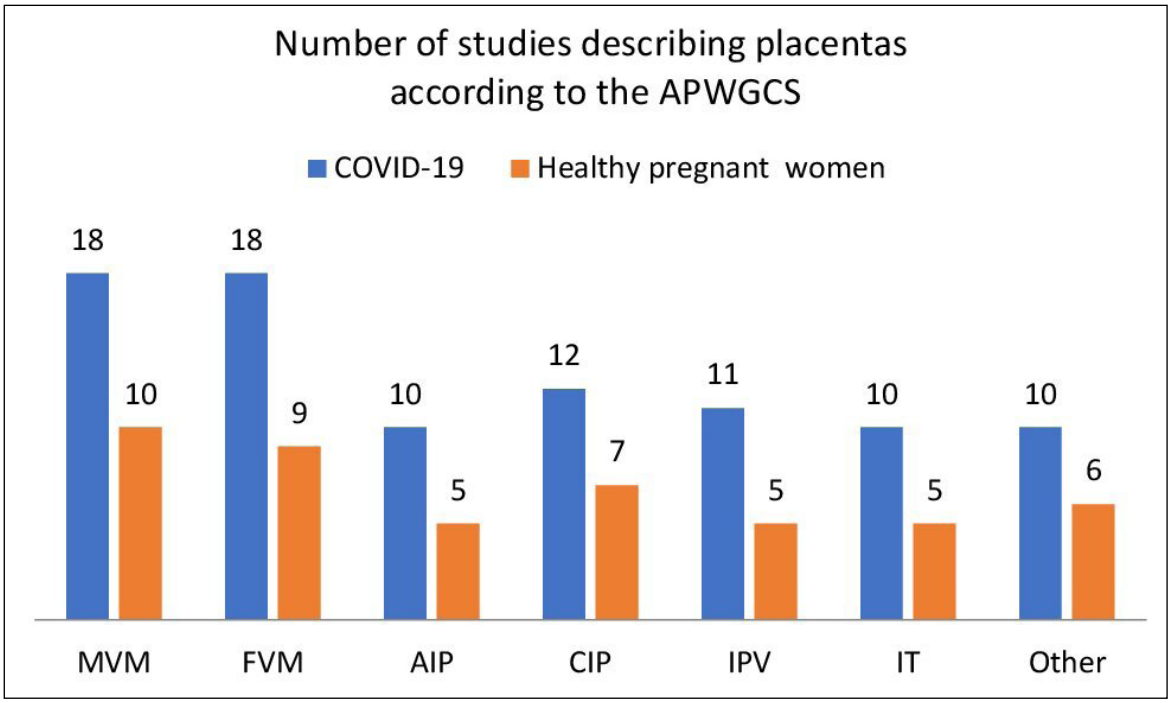

Fig. 2. Number of studies describing placentas according to the Amsterdam Placental Workshop Group Consensus Statement.
Note: MVM – maternal vascular malperfusion, including central villous infarction, peripheral villous infarction, villous agglutination, accelerated villous maturation, decidual arteriopathy (any atherosis and fibrinoid necrosis, mural hypertrophy of membrane arterioles, or absence of spiral artery remodeling), or retroplacental hematoma; FVM – fetal vascular malperfusion, including clustered avascular villi, fetal vessel mural fibrin, delayed villous maturation, hypercoiled umbilical cord, or chorioangiosis; AIP – acute inflammatory process, including maternal or fetal inflammatory response; CIP – chronic inflammatory process, including chronic villitis or low-grade chronic deciduitis with plasma cells; IPV – Increased perivillous fibrin; IT – Intervillous thrombosis. OTHER – including umbilical cord abnormalities, chorangiosis, hypertrophic arteriolopathy, subchorionic thrombus, a basal plate with attached myometrial fibers, microscopic accretism, villous edema, increased circulating nucleated red blood cells, or membranes with hemorrhage.

cations in the mechanisms of formation of multiple organ failure syndrome, such variants of the pathohistological characteristics of the placental complex as maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), severe perivillous fibrin deposition (IPV) were identified for analysis, intervillous thrombus formation (IT). The category "other" - "OTHER" included umbilical cord anomalies, chorangiosis, hypertrophic arteriolopathy, edema of the villous space, hemorrhage into the fetal membranes, etc. Separately, inflammatory changes in the placental complex were described, which reflect the inflammatory response of the maternal body and intrauterine fetus (chorioamnionitis, chronic deciduitis, chronic villitis, etc.).

As for the results of immunohistochemical studies of "covid" placentas, they were presented in 24 of the 30 full-text papers selected for analysis (see Flowchart).

The main group of women who had COVID-19 during this pregnancy (pregnant women with a "Covid" history) consisted of 2581 participants; the control group included 2033 apparently healthy pregnant women who did not have any indication of SARS-CoV-2-associated acute respiratory viral infection (ARVI) or placentas that were studied before the COVID-19 pandemic.

The number of participants whose placentas were described "quantitatively", i.e. those where morphological and histological changes are described as a percentage, respectively, for the main and control groups were 2401 and 1910 (Table 3)

The distribution of the number of studies by type of study is presented in Table 4.

The most common pathological histological patterns of the placenta in the group of women with a "covid history" include, according to most of the studies presented, maternal vascular malperfusion (MVM) of the placental complex, the most recognizable pattern of placental damage associated with altered uterine and intervillous blood flow (Table 5).

Thus, MVM was detected 1.7 times more often ($t = 2.72766$, $p = .000447$, $RR = 5.29$, $OR = 7.98$.) than in healthy women; indications of these histological findings in placentas were described in 18 studies for the COVID-19 group, and in 10 studies for the control group. Significantly more often, 1.8 times ($t = 2.19492$, $p = .019041$), there was increased deposition of fibrinoid in the villous, perivillous and intervillous space of the placenta - (IPV - increased

Table 2 . General characteristics of the studies included in the systematic review

Author	Country	Study design	COVID-19 group	Control group	Conclusion
Adam A.M. et al.	Romania, 2023	**PCCS**	26	26	IHC analysis failed to demonstrate positivity for CD19, CD3, CD4, CD8, and CD56 markers. Immunophenotyping analysis could be useful for risk stratification of pregnant patients, while further studies are needed to determine the extent of immunological decidual response in patients with various forms of COVID-19.
Bertero L. et al.	Italy, 2021	**CCS**	11	58	Inflammatory and thrombo-hemorrhagic alterations were the most frequent and peculiar pathological changes we observed in our series compared to control samples, but only chronic villitis/VUE and accelerated villous maturation retained statistical significance after restricting the analysis to placentas delivered after a previous CS.
Boyraz B. et al.	USA, 2022	**RS**	67	126 GBS-colonized	Cases of COVID19 are covered patients' placentas and found no placentas with SARS-CoV-2 placentitis or evidence for viral infection by IHC. ... exposure to SARS-CoV-2 during pregnancy is associated with increased MVM and a trend toward associated increased FVM pathologies, which suggests that SARS-CoV-2 viral infection during pregnancy, even cleared, can have lasting effects on the placenta.
C. Tasca et al.	Italy, 2021	**PMCCS**	64	64	The presented evidence suggests that SARS-CoV-2 infection during the third trimester does not affect placental histology and morphology, nor causes obstetrical or fetal adverse outcomes.
Celik E. et al.	Turkey, 21	**PMCS**	31; 22 (73%) asymptomatic/ mild; 8 (27%) -moderate to severe/critical disease	NP	Placental inflammation can occur in mothers with SARS-CoV-2 infection. The disease severity is associated with ischemic placental pathology which may result in adverse pregnancy outcomes such as PTL and IGR. A high maternal inflammatory state is possibly contributing to placental insufficiency. The frequency of placental abnormalities is correlated with the severity of disease.
Corbetta-Rastelli C.M. et al.	CA, 2023	**DRCS**	131; mild - 60%.	138; 8 - twins	There was a higher frequency of placental features associated with response to infection before 20 weeks of gestation than that from infections after 20 weeks of gestation (P=.001). ... features of severe MVM were only found in the placentas of patients with SARS-CoV-2 infection in the 2d and 3d trimesters of pregnancy, not in the placentas of patients with COVID-19 in the 1st trimester. Placentas from patients with COVID-19 showed no specific pathologic feature, regardless of the timing or severity of the disease. There was a higher proportion of placentas from patients with COVID-19–positive tests in earlier gestations with evidence of placental infection–associated features.
Damman E. et al.	France, 2023	**RS**	96	NP	Traditional risk factors such as diabetes and obesity were not associated with severe placental damage. However, our results show that severe placental damage was significantly associated with IUGR, maternal hypofibrinogenemia and thrombocytopenia.
Edlow A.G. et al.	Boston, USA. 2020	**PCS**	44	44	This report of maternal viral load, transplacental antibody transmission, and placental pathology in 127 pregnancies during the SARS-CoV-2 pandemic provides needed data about maternal viral control, reduced transplacental transfer of anti–SARS-CoV-2 antibodies, and lack of vertical transmission in mother-neonate dyads.
Facchetti F. et al.	Italy, 2020	**CSS**	15	34	The detection of SARS-CoV-2 in the placenta of COVID-19, distributed in multiple fetal cell types, including circulating cells, combined with the failure to reveal SARS-CoV-2 S-protein expression, establishes the infrequent occurrence of mother-to-fetus SARS-Cov-2 transmission with adverse effects on the newborn.
Ferraz T. et al.	Canada, 2023 - 13 studies from 13 countries	**SR**	500	301	Maternal obesity and antenatal infection with SARS-CoV-2 influence placental health and together, could lead to worse outcomes for babies.
Gao L. et al.	China, 2021	**CSS**	8	NP	We found no evidence of vertical transmission and adverse maternal-fetal outcomes in the placentas of third trimester COVID-19 pregnancy women, which provided further information for the clinical management of those women in the third trimester. However, further studies are still needed for patients with infections in different stage of gestation, especially in first and second trimester.

Table 2. continuation

Giordano G. et al.	Italy	**CR**	5	Yes	Placental patterns in pregnancy due to COVID-19 in the late stage of gestation indicate no evidence of vertical trans-placental SARS-CoV-2 transmission or a significant impact on the perinatal outcome of newborns, in both mild and more severe cases.
Girolamo R. et al.	Italy, 2021	**SR** &**MA** (56 studies)	1008	Yes	...A large proportion of pregnancies complicated by SARSCoV-2 infection present placental histopathologic abnormalities consistent with placental inflammation and hypoperfusion, whereas approximately 17.5% of these pregnancies did not show any placental anomalies. Subgroup analyses according to the presence of maternal symptoms or high-risk pregnancy showed similar results with most placentas from women with SARS-CoV-2 infection in pregnancy
Glynn S.M. et al.	USA, 2022	**PCS**	90 Nonacute SARS-CoV-2 (N = 64); Acute SARS-CoV-2 (N = 26)	188	The frequency of FVM lesions is significantly higher among the placentas from women with acute SARS-CoV-2 infection compared with women with nonacute SARS-CoV-2 infection at delivery. These findings raise new questions regarding the etiology, clinical consequences, and potential downstream effects of FVM lesions in the setting of acute SARS-CoV-2 infection.
Gychka S.G. et al.	Ukraine, 2022	**CCS**	85; 68% - mild disease; 32% - severe disease	126	Placental vascular walls thickened and the lumen narrowed in women who gave live birth but contracted COVID-19 during pregnancy. Smooth muscle proliferation and collagen fiber deposition appear to play major roles in the development of placental vascular remodeling. Elucidating the mechanism of these events should help provide new therapeutic targets to combat COVID-19, and prevent stillbirth and premature birth, as well as alterations of child development.
Horn L.C. et al.	Germany, 2022	**CR**	2	NP	Sudden intrauterine death may occur in mothers who are oligosymptomatic for COVID-19. Acute placental failure is responsible for SIUD, demonstrated by massive perivillous fibrin deposits and extensive necroses of the villous trophoblast with SARS-CoV-2-positivity based on IHC staining and RT-PCR.
Hsu A.L. et al.	USA, 2021	**CR**	1	NP	This is the 1st report of placental COVID-19 despite mild COVID-19 disease in pregnancy: SARS-CoV-2 was found in the placenta, but the newborn was COVID-19 negative. Our case shows maternal vascular malperfusion, with no features of fetal vascular malperfusion. Evidence of placental COVID-19 raises concern for placental vasculopathy and potential vertical transmission.
Husen M.F. et al.	The Netherlands, 2021	**PMCS**	36 (33/3 twins); Active COVID-19 (N = 17) Resolved COVID-19 (N = 19);	NP	We described a unique placental signature with a specific presence of CD20+ B-cells clustering around the necrotic syncytiotrophoblast combined with chronic intervillositis and perivillous fibrin depositions, in pregnant patients with COVID-19 not seen in historic controls. We assume foetal well-being can potentially be threatened by an increased risk of histopathologic placental abnormalities resulting from a localised immunological response triggered by the presence of SARS-CoV-2 in the placenta. …We recommend SARS-CoV-2 PCR of maternal blood and vaginal fornix, to identify women at risk of placental infection with SARS-CoV-2 and to prevent negative pregnancy outcomes due to COVID-19.
Jaiswal N. et al.	India, 2021	**PAS**	27	27	Asymptomatic or mildly symptomatic SARS-CoV-2 positive pregnant women, with otherwise uncomplicated pregnancies, show evidence of placental injury at a microscopic level. The features of MVM, FVM and other features of placental inflammation are found more in the SARS-CoV-2 affected women when compared to controls. Although this dysfunction does not seem to result in poor fetal outcomes but may have adverse long term effects. This is an evidence that can't be ignored. A systemic nature of the disease and its ability to trigger a procoagulant state in the body may be responsible for the placental injury.
Kosič N,	Slovenia, 2023	**CR**	1	NP	Based on clinical, pathological, and microbiological correlation supported by specific histological findings and IHC staining, we conclude that the cause of fetal death in a woman with mild Covid-19 disease was acute placental failure triggered by SARS-CoV-2 infection.
Levitan D. et al.	USA, 2021	**CSS**	65	85	Our study results and a literature review suggest that there is no characteristic histopathology in most placentas from women with SARS-CoV-2 infection. Likewise, direct placental involvement by SARS-CoV-2 is a rare event.

Table 2. continuation

Linehan L. et al.	Ireland, 2021	**CR**	1	5	We present a case of third trimester pregnancy complicated by SARS-CoV-2 infection and subsequent reduced fetal movements, resulting in emergency CD with demonstrable placental SARS-CoV-2 placentitis. SARS-CoV-2 placentitis therefore appears to be an uncommon but distinctive complication of maternal COVID-19 infection and appears to have the potential to cause significant placental injury, potentially resulting in fetal compromise.
Marton T. et al.	UK, Birmingem, 2021	**CR**	1	NP	This case report supports maternal- fetal vertical transmission of SARS-CoV-2 virus. Our case gives insight in the morphology of SARS-CoV-2 placentitis that is observed in the most severe COVID-19 related pregnancy complication leading to intrauterine fetal death. The main components of SARS-CoV-2 placentitis are MPFD and CHI. IHC and ISH proved the vertical transmission of the virus infecting the chorionic epithelium of the placenta.
Rakheja D. et al.	USA, 2022	**CSS**	9	NP	We have described an IHC assay for SARS-CoV-2 nucleocapsid protein that is highly sensitive, specific, and robust, allowing routine use in placental pathology practice.
Rebutini PZ. et al	Brazil, 2021	**PCCS**	19 (10 - asymptomatic, 9 - symptomatic)	19 (gave birth before the pandemic)	Pregnant women with symptomatic SARS-CoV-2 infection are more likely to exhibit an adverse fetal outcome, with slightly more frequent histopathologic findings of MVM and FVM, and CHI. The morphometric changes found in the placentas of the COVID-19 group do not seem to be different from those observed in the Control group... Only the deposition of villous fibrin could be more accentuated in the COVID-19 group ($p = 0.08$ borderline)
Şahin O. et al.	Turkey, 2022	**PCCS**	30 - with asymptomatic COVID-19	30	The results demonstrated that asymptomatic COVID-19 had no significant effect on pregnancy and neonatal complications. However, mural hypertrophy in the placenta was found at a significantly higher rate in pregnant women with asymptomatic COVID-19. Although concerns about placental vasculopathy increase in COVID-19-positive pregnant women...
Sharps M.C. et al.	United Kingdom, 2022	**CCS**	28: 8-activ COVID-19; 20 – post-COVID-19	16	…There was no specific placental pathology associated with maternal SARS-CoV-2 infection during pregnancy. The observed increase in the number of placental macrophages warrants further investigation to see if these cells are responding to any possible maternal hypoxia affecting the placenta (which increases cytokine release from placental tissue).
Smithgall MC,	USA, 2020	**CSS**	51	25	In our limited study of 51 placentas from SARS-CoV-2-positive women in the third trimester, ISH and IHC showed no definite evidence of SARS-CoV-2 in the placentas, and we noted non-specific histomorphologic changes suggestive of maternal–fetal vascular malperfusion. All neonates tested negative for SARS-CoV-2, and all women recovered clinically. Further studies, including more sensitive techniques for viral infection (e.g. RT-PCR), are warranted.
Surekha M.V. et al.	India, 2023	**CSS**	122 -seropositive for SARS-CoV-2 IgG, but none reported COVID-19 history	90 (of the 212, 63.2% (n=134) women were anemic)	Although the prevalence of anemia was high in the present study, its effects on placentae were less prominent than that of SARS-CoV-2. The most intriguing and novel finding of the study was strong evidences that of maternal COVID-19 infection, which was otherwise asymptomatic, was being associated with increased placental damage, indicating histopathological features of placental hypoxia and thus possibilities of intrauterine fetal hypoxia.
Taglauera E.S. et al.	USA, 2021	**CCS**	16	8	These data suggest that in acute maternal SARS-CoV-2 infections, decreased placental ACE-2 protein may be the result of ACE-2 shedding. Overall, this work highlights the importance of ACE-2 for ongoing studies on SARS-CoV-2 responses at the maternal-fetal interface.
Watkins J.C. et al.	USA, 2021	**RS**	7 (6 from live-born neonates; 1- stillbirth.	NP	SARS-CoV-2 placentitis is defined by the triad of histiocytic intervillositis, perivillous fibrin deposition, and trophoblast necrosis. The features may occur in cases without confirmed transplacental transmission. The damage caused by SARS-CoV-2 placentitis is likely mediated by complement activation.

Note: ACE – angiotensin converting enzyme; CHI – Chronic Histiocytic Intervillositis; CR – case report studies; CCS – case control studies; CD – caesarean delivery; CSS – cross-sectional studies; DRCS – descriptive retrospective cohort study; FVM – fetal vascular malperfusion; GBS-colonized – group B streptococcus colonized; IGR –intrauterine growth retardation; IHC – immunohistochemical analysis; ISH - in-situ hybridization; MPFD – Massive Perivillous Fibrin Deposition; MVM - maternal vascular malperfusion; NP – not presented; PAS – prospective analytical study; PCS – prospective cohort study; PCCS – prospective, case-control study; PMCS – Prospective multicentre cohort study; RS – retrospective study; PTL – preterm labor; RT-PCR – Real-time polymerase chain reaction; SR – systematic review; SR&MA – systematic review and meta-analysis.

Table 3. Morphro-histological patterns of placentas in women whore covered from COVID-19 during pregnancy and in apparently healthy women (studies with a "quantitative" description of morphological and histological changes in the placenta)

Authors	Number of placentas examined		MVM, n / %		FVM, n / %		AIP, n / %		CIP, n / %		IPV (Increased perivillous fibrin), n / %		IT (Intervillous thrombosis), n / %		OTHER, n / %		NO patjology, n / %	
	COVID-19	Control	COVID-19	Control	COVID-19	Control	COVID-19	Control	COVID-19	Control	COVID-19	Control	COVID-19	Control	COVID-19	Control	COVID-19	Control
Boyraz B. et al.	67	126	(-)	(-)	11 /16,4%	(-)	(-)	(-)	14/20,9%	(-)	(-)	(-)	7/10.4%	(-)	4 /6%		NP	NP
C. Tasca C. et al.	64	64	17/26,6%	11 /17,2%	5 /7,8%	12 /18,8%	2/3,1%	1 / 1,6%	10/15,6%	7/10,9%	(-)	(-)	(-)	(-)	(-)	(-)	21/ 32.8%	27/42.2%
Corbetta-Rastelli C.M. et al.	131	0	83 /64%	(-)	16 /12%	(-)	37 /28%	(-)	19 /15%	(-)	(-)	(-)	(-)	(-)	(-)	(-)	30/ 23%	NP
Damman E. et al.	96	0	29 /15,5%	(-)	5 /2,4%	(-)	32 /16,7%)	(-)	16 /8,3%	(-)	(-)	(-)	(-)	(-)	(-)	(-)	101 /53,5%	NP
Edlow A.G. et al.	44	44	16 / 36%	8 / 18%	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	28 / 64%	NP
Ferraz T. et al.	500	301	314/ 62,85%	(-)	244 /48,7%	(-)	(-)	(-)	426 /85,2%	(-)	430 /86%	(-)	(-)	(-)	(+)	(-)	NP	NP
Gao L. et al.	8	0	8 / 100%	(-)	(-)	(-)	(-)	(-)	2 / 25%	(-)	7 /87,5%	(-)	(-)	(-)	2 /25%	(-)	NP	NI
Giordano G. et al.	**5**	0	4 /80%	(-)	4 /80%	(-)	1 /20%	(-)	5 /100%	(-)	(-)	(-)	3 /60%	(-)	(-)	NP	NP	NP
Girolamo R. et al.	**1008**	895	309 /30,7%	(-)	273 /27,08%	%	(-)	(-)	258 /25,65%	(-)	329 /32,7%	(-)	147 /14,6%	(-)	378 /37,5%	(-)	176 /17,5%	NP
Glynn S.M. et al.	**90**	188	25 /27,95%	45 / 24,1%	32 /36,05%	25 /13,2%	9 /9,71 %	12 /6,38%	9 /9,71%	13 / 6,9%	(-)	(-)	19 /21,5%	27 /14,4%	4 /4,45%	16 /8,35%	NP	NP
Horn L.C. et al.	**2**	0	(-)	(-)	(-)	(-)	(-)	(-)	2 /100%	(-)	2 /100%	(-)	(-)	(-)	(-)	(-)	NP	NP
Husen M.F. et al.	36	0	5 /12,82%	(-)	4 /10,26%	(-)	6 /15,38%	(-)	4 /10,26%	(-)	4 /10%	(-)	(-)	(-)	4 /10,26%	(-)	14 /39%	NP
Jaiswal N. et al.	**27**	27	10 /36,68%	3 /12,58%	7 /24,67%	2 /7,4%	8 /29,6%	3 /11,1%	(-)	(-)	26 /96,2%	6 /22%	3 /11,1%	(-)	5 /18,5%	1 /3,7%	NP	NP
Kosič N. et al.	**1**	0	(+)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(-)	NP
Levitan D. et al.	**65**	85	23 /35%	31 /36%	6 /9%	16 /19%	(-)	(-)	23 /35%	29 /34%	10 /15%	17 /20%	15 /23%	19 /22%	16 /25%	32 /38%	NP	NP
Rebutini PZ. et al	**19**	19	11 /56,6%	3 /15,7%	15 /78,9%	5 /26,31%	(-)	(-)	16 /84,21%	17 /89,5%	7 /36,8%	1 /5,26%	6 /31,05%	1 /5,26%	15 /78,9%	14 /73,7%	NP	NP
Şahin O. et al.	**30**	30	25 /83,3%	9 /30%	13 /43,3%	8 /26,7%	1 /3,3%	2 /6,7%	(-)	(-)	23 /76,7%	17 /56,7%	2 /6,7%	1 /3,3%	6 /20%	5 /16,7%	NP	NP
Sharps M.C. et al.	**28**	16	16 /57%	6 /34,5%	6 /21,4%	4 /25,5%	2 /6%	(-)	(-)	1 /6%	(-)	(-)	(-)	(-)	(-)	(-)	NP	NP
Smithgall M.C. et al.	**51**	25	17 /33,3%	9 /36%	9 /17,7%	3 /12%	(-)	(-)	2 /3,9%	2 /8%	(-)	(-)	8 /15,7%	7 /28%	14 /28%	2 /8%	NP	NP
Surekha M.V. et al.	122	90	47 /38,55%	14 /15,95%	68 /55,4%	38 /42,3%	77 /63,3%	36 /39,6%	17/14,25%	2 /2,75%	49 /40,3%	33 /36,3%	NP	NP	NP	NP	NP	NP
Watkins J.C. et al.	**7**	0	2 /28,57%	(-)	3 /42,85%	(-)	1 /14,28%	(-)	6 /85,7%	(-)	6 /85,7%	(-)	1 /14,28%	(-)	(-)	NP	NP	NP
Total	2401	1910	961 / 38,51%	139 / 7,27%	721 / 28,9%	113 / 5,91%	176 / 7,05%	54 / 2,82%	829 / 33,22%	71 / 3,71%	893 / 35,79%	74 / 3,87%	211 / 8,45%	55 / 2,87%	448 / 17,95%	70 / 3,66%	376 / 15,07%	27/ 42.2%

Note: AIP – including acute maternal or fetal inflammatory response; CIP – including chronic villitis or low-grade chronic deciduitis with plasma cells; IPV – increased perivillous fibrin; IT – intervillous thrombosis; MVM – maternal vascular malperfusion, including central villous infarction, peripheral villous infarction, villous agglutination, accelerated villous maturation, decidual arteriopathy (any atherosis and fibrinoid necrosis, mural hypertrophy of membrane arterioles, or absence of spiral artery remodeling), or retroplacental hematoma; FVM – fetal vascular malperfusion including clustered avascular villi, fetal vessel mural fibrin, delayed villous maturation, hypercoiled umbilical cord, or chorioangiosis; OTHER – including umbilical cord abnormalities, chorangiosis, hypertrophic arteriolopathy, SCT - subchorionic thrombus, a basal plate with attached myometrial fibers, microscopic accretism, villous edema, increased circulating nucleated red blood cells,or membranes with hemorrhage; NP - not presented; (+) – the description is narrative, signs are present; (-) – narrative description, no signs.

Table 4. Distribution of participants by type of study

Type of study	Number of study	Participants	
		COVID-19, n / %	Control, n / %
Case control study	4	141 / 5.9%	129 /6.75%
Case report	3	8 / 0.33%	0/
Cross sectional study	4	246 / 10.24%	200 / 10.47%
Descriptive retrospective cohort study	1	131 / 5.45%	0
Prospective analitycal study	1	27/ 1.12%	27 / 1.41%
Prospective cohort study	3	170 / 7.08%	232 / 12.14%
Retrospective study	3	170 / 7.08%	126 /6.59%
Systematic review	2	1508 / 62.8%	1196 / 62.61%
Total	21	2401	1910

Table 5. Comparative frequency of pathological histological patterns of the placenta in pregnant women who have recovered from COVID-19 and in healthy pregnant women.

Group / Indicator	COVID-19 group	Control group	P value (at p < .05)	CI
MVM, M ± m (SD), %, size 18 – 10	50.32 ± 25.29	30 ± 24.87	t = 2.72766. p =.00447	t = 4732, 95% CI 2869.86 - 6594.14
FVM, M ± m (SD), %, size 18 – 9	39.29 ± 26.84	28.28 ± 21.76	t = 1.4791 p = .072893	t = 1101, 95% CI-1028.38 - 3230.38
AIP, M ± m (SD), %, size 10 – 5	23.63 ± 20.83	30.1 ± 26.45	t = -0.72423 p=.237578	t = 2062, 95% CI -624.98 - 4748.98.
CIP, M ± m (SD), %, size 12 – 7	45.65 ± 33.67	35.38 ± 38.04	t = 0.83874 p =.203737	t = 1027, 95% CI -2374.63 - 4428.63
IPV, M ± m (SD), %, size 11 – 5	58.5 ± 29.43	33.12 ± 20.83	t = 2.19492 p =.019041	t = 2727, 95% CI -425.45 - 5879.45
IT, M ± m (SD), %, size 10 – 5	29.89 ± 22.66	21 ± 12.79	t = 1.03104 p=.156401	t = 777, 95% CI -1606.71 - 3160.71
Other, M ± m (SD), %, size 10 – 6	30.12 ± 25.91	35.55 ± 30.31	t = -0.50656. p = .308367	t = 543, 95% CI -2509.69 - 3595.69

Note: MVM – maternal vascular malperfusion, including central villous infarction, peripheral villous infarction, villous agglutination, accelerated villous maturation, decidual arteriopathy (any atherosis and fibrinoid necrosis, mural hypertrophy of membrane arterioles, or absence of spiral artery remodeling), or retroplacental hematoma; FVM – fetal vascular malperfusion, including clustered avascular villi, fetal vessel mural fibrin, delayed villous maturation, hypercoiled umbilical cord, or chorioangiosis; AIP – acute inflammatory process, including maternal or fetal inflammatory response; CIP – chronic inflammatory process, including chronic villitis or low-grade chronic deciduitis with plasma cells; IPV – Increased perivillous fibrin; IT – Intervillous thrombosis. OTHER – including umbilical cord abnormalities, chorangiosis, hypertrophic arteriolopathy, a basal plate with attached myometrial fibers, microscopic accretism, villous edema, increased circulating nucleated red blood cells, or membranes with hemorrhage. Size – number of studies that quantify placental patterns in pregnant women who have recovered from COVID-19 and in pregnant controls.

perivillous fibrin or fibrinoid), which may be due to hypercoagulation or degenerative processes. According to characteristics such as FVM, i.e. placental lesions associated with reduced or absent perfusion of the villous parenchyma of the fetus, as well as the frequency of intervillous thrombosis (IVT - Intervillous thrombosis), associated with the leakage of fetal blood into the intervillous space and further blood clotting, there were no significant differences between the groups. However, analysis of the probability and chance of developing FVM (RR = 4.88, OR = 6.46), acute (RR = 2.5,

Table 6. Course of pregnancy, perinatal outcomes and results of immunohistochemical study of "COVID-19" placentas.

Author	COVID-19 gruop	Control group	Maternal outcomes	Fetal outcomes	Gestational age at delivery	Comorbiduty	Complications of pregnancy	Immunohistochemical analysis of placenta
Adam A.M. et al.	26	26	PTL - 5 (19.2%) vs 2 (7.7%); OR 0.98 (0.96–1.00) p 0.22	Weight, g - 3151.92 ± 564.70 vs 3189.23 ± 406.08; AS - 5 min - 8.38 ± 0.63 vs 8.88 ± 0.58, OR 4.11 (1.211–13.974), p - 0.005; NICU -1 (3.8%) vs 0 (0%) OR 1.01 (0.98–1.05) 0.31	38.19 ± 1.91; 38.53 ± 1.10	DM- 1- 3,8% vs 0%, p - 0.31; Thrombophilia 4 (15.4%) VS 2 (7.7%), p-0.38; Lower limb varicose veins- 2 (7.7%) VS 1 (3.8%), p- 0.55	IGR 4 (15.4%) vs 2 (7.7%) OR 0.32 (0.03–3.12) p - 0.38	IHC demonstrate positivity for CD19, CD3, CD4, CD8, and CD56 markers
Bertero L. et al.	11	58	NP	1 neonatal nasopharyngeal swab was positive for the virus	4 - PTL, 6 - full-term, 1-twins (35 (30–38)	β-thalassemia - 2; CH -1, severe obesity (BMI: 50)-1, GD-1,	Polyhydramnios -1; Intrahepatic cholestasis of pregnancy -1	NP
Boyraz B. et al.	67	126- GBS-colonized	20 (29.8%) including tight/ wrapped nuchal cord, shoulder dystocia, and failure to progress; GBS.	Weight, g, 993-4555; 3202.1 ± 538.7	34.0 to 41.5 (mean = 39.0 weeks)	33 (49.2%) - GH, GDM, GBS infection, obesity, breast cancer, acute kidney injury, anemia, Coxsackie virus infection, Zika virus exposure, depression		SARS-CoV-2 spike (S) protein expression placentas
C. Tasca C. et al.	64	64	VD - 42 (65.6%) 35 (54.7%), CD - 20 (31.2%) 26 (40.6%), Vacuum-assisted VD - 2 (3.1%) 3 (4.7%)	Weight (g) - 3160.6 ± 449.4 3237.4 ± 479.5 0.309 AS at 5 minutes <7 0 (0%) 0 (0%) 1; NICU admission 1 (1.6%) 3 (4.7%) 0.310	≥34 weeks	ICP, GH, thyroid diseases, GDM	IUGR, PTL, PPH	NP
Celik E. et al.	31; 22 asymptomatic/ mild; 8 -moderate to severe/critical disease	NP	NP	AS - 5' less than 7, n (%) 1 (5)vs 2 (25); CNAO, , n (%) 2 (9)vs 3 (37) 0.09; SGA , n(%) 1vs 2; NICU admission, n(%) 3 (14) 4 (50) 0.06; Prematurity (CPAP), n(%) – 2 (25)	38.6 (37.9–38.8) vs 38.1 (31.8–38.8) 0.37	Comorbid conditions, 3 -(13.6%) vs 5 (62.5) p 0.02*;DM 1 vs1; Hypothyroidism 1vs 2; Asthma – 0 vs1;Epilepsy – 0 0vs1;	GDM - n (%) 2 (9) vs 2 (25); GH, n (%) – 0 vs 2 (25); GC, n (%) 1 (5) vs 0;	SARS-CoV-2 spike (S) protein expression placentas
Corbetta-Rastelli C.M. et al.	131	138, 8 -twins	CD - 54 (41.0); PTL - (<37 wk), n (%) 131 35 (27.0); Required blood transfusion-7 (5.3%); PA - 2 (1.5%); Umbilical cord or placental anomalies - 5 (3.8);	NICU admission, n (%) 135 47 (35.0); Composite neonatal morbidity, n (%) - 139 - 51 (37.0%); 10% - FGR; 9% - clinical CA; SGA n (%) 136 - 11 (8.0%);	38.6 (37.0–39.0)	BMI of >30 kg/m2 (n=55 [50%]), asthma (n=20 [15%]); CKD - 1 (0.8); IVF pregnancy-7 (5.3);	GH- 21, PE without severe features, 7- PE with severe features-15 , GDM - 20	NP
Damman E. et al.	96	NP	CD -mild covid - 28 (33%) vs severy- 8 (67%); PTL - 15 (18%) vs 11 (92%) <0.001; IUFD 1 (1.2%) vs4 (33%) <0.001; IUGR 7 (8.3%) 5 (42%) <0.01;	13 neonates (13.5%) had IUGR and 26 (27%)- PTL; 5 cases of IUFD. 24 neonates (25.0%) - NICU;	36 + 6 days	BMI - 25.8 (±6.0) kg/m2 and 18 patients (18.8%) had obesity.	14- (14.6%) - GD; PE 2 (2.4%); Anemia mild COVID - 51 (67%) vs severy - 4 (33%);	IHC - with anti-SARS-CoV-2 NcP performed in all 12 cases of severe placental damage; SARS-CoV-2 PCR tests - on extracted RNA in 9/12 cases and were all positive.
Edlow A.G. et al.	44	44	Composite morbidity - 2 (3) vs 9 (14) p=.03; CD - 39 (62)vs 21 (37); PPL - 5 (8) vs10 (18) .11; Spontaneous 2 (40) vs 3 (30) NA;	Composite morbidity - 5 (8) vs 14 (25) p=.01; FGR -1 (2) vs 4 (6) .06; ND -0 vs 2 (4), p= .16;	39.1 (38.3-39.7) 39 (37.4-40.1) .14;	CH- 1 (2) vs 3 (5), p .32; DM or GDM - 12 (19) vs 11 (17) .79; BMI >30 18 (29) 26 (41) .15; Asthma 8 (13) 7 (11).76; Thyroid disease 4 (6) 13 (21) .02; Cancer 2 (3) 2 (3) .99;	PE/ GH: 13 (21) vs 15 (26)	Placenta examined for expression of the SARS-CoV-2 receptor (ACE2) and the TMPRSS2; identified membranous syncytiotrophoblastic ACE2 expression.

Table 6. continuation

Facchetti F. et al.	15	34	Labor Induction - 5; TCP--1, PPH-2, PPP- -1, ARDS-2,CA - 1	NICU - 4; Severe neonatal morbidity: RDS - 1, HMD -2, . NEC - 1, NI-2, PI - 1. CH	32+6 - 41+3	HYT, CHB - chronic HBV hepatitis, OB - 4, ATH --1, HbC - 1; COL: cholestasis -1, CH -1	NP	IHC for the SARS-CoV-2 S-protein; for the inflammatory cells, Hofbauer cells, platelets and megakariocytes. Very few lymphoid T-cells (A, immunostain for CD3), B-cells (B, for CD20) occurred in the placenta.
Ferraz T. et al. -	CS -7 (participants -49); Cohort Series – 6 (participants - 451 COV+; 301 - COV-;	451	NP	Only weght	At term	Obesity-BMI ≥ 25.0 kg/m2 before conception	NP	NP
Gao L. et al.	8	NP	CD - 7; Indication for delivery - SARS-CoV-2;	AS -8-10; wegth - 2,17-3,55	3+6 -40+1	no	NP	IHC for inflammatory cells and Hofbauer cells, (SARS) CoV-2 RNA FISH and SARS-CoV-2 NcP. The list of primary antibodies was as follows: anti-CD3, anti-CD20, anti-CD163, anti-CD68, anti-CD138.
Giordano G. et al.	5	yes	At term	None of the newborns had signs or symptoms related to COVID-19, stayed with their mother (rooming-in), receiving breast milk, and were discharged on the 3rd day of life	Late pregnancy	GDM 1, HYT - 2.	(+)	Anti-CD3, anti-CD8, anti-CD20, anti-CD68 antibodies and CD15
Girolamo R. et al.	1008	да	NP	(+)	NP	Subanalyses of symptomatic women only and those with a high-risk pregnancy	(+)	(+)
Glynn S.M. et al.	97 - 90 placentas; Nonacute SARS-CoV-2 (N = 64); Acute SARS-CoV-2 (N = 26)	SARS-CoV-2 Negative (N = 188)	CD 20 (31.2%); 8 (30.8%); 95 (50.5%)	SGA, n (%) 11 (17.2); 2 (7.69); p= 0.333 37 (19.7);p=0.328; Weight, (g) 3082 ± 627 3190 ± 631 0.457 3048 ± 680 0.595; 5-minute AS, median (IQR) 9.00 [9.00-9.00] 9.00 [9.00-9.00] 0.604	37+2 - 39+6	DM, n (%) 2 (3.12); 0 (0.00);p >0.99; 8 (4.26);p= 0.880;	PE/GH, n (%) 5 (7.81) 3 (11.5) 0.686 33 (17.6) 0.132; GDM, 3 (4.69) 0 (0.00) 0.554 16 (8.51) 0.260; CA, 2 (3.12) 1 (3.85) >0.99 13 (6.91) 0.650;	SARS-CoV-2 spike NcP expression placentas
Gychka S.G. et al.	85	126.	68% - mild disease; 32% - severe disease accompanied by pneumonia	AS, 7.9 ± 0.4 7.7 ± 0.8	39+6	NP	Uncomplicated	Antibody against α-smooth muscle actin and Kv11.1 channel, was carried out.
Horn L.C. et al.	2	2- oligosymptomatic for COVID-19	NP	Sudden IUD in the 2-nd trimester; The female fetus of 730 g (20th percentile) and 24.4 cm crown-rump length (< 10th percentile). No malformations nor inflammatory changes were detected on autopsy.	15 + 2 and 27 + 3 weeks	NP	NP	IGH staining was strong and diffusely positive for SARS-CoV-2 in the villous trophoblast and rarely within the villous stromal cells. Placental SARS-CoV-2 infection was confirmed by RT-PCR.

Table 6. continuation

Hsu A.L. et al.	1	VD	NP	Newborn course was uneventful; AS of 8 and 9 at 1 and 5 minutes; Weight -3521 g (66th percentile); discharged home at 36 hours of life,	40-4/7 weeks for labor induction	NP	Uncomplicated	IHC was performed with SARS-CoV-2 - NcP specific rabbit monoclonal antibody; To identify trophoblasts, IHC was performed using rabbit recombinant anti-cytokeratin 7 (CK7) monoclonal antibody
Husen M.F. et al.	36 (33/3 twins); Active COVID-19 (n = 17) Resolved COVID-19 (n = 19);	NP	Maternal condition for emergency CD - 5 (13.9); Labour by induction 10 (27.8%); Spontaneous labour 1 (2.8); CD - 22 (61.2); Vaginal instrumental 1 (2.8) .	Breech position 3 (8.3); FD 7 (19.4) - for CS; SGA (BW < 10th percentile) 9 (23.1); AS <7 at 5 min, n (%) 5 (12.8); pH < 7.10, n (%) 2 (5.1) ;	PTL, n (%) total- 11 (30.6%) 9 (52.9) 2 (10.5) 0.01;	Comorbidities, n = 34 (%); Autoimmune disease total- 3 (8.3%); 1 (5.9%); 2 (10.5%) 0.88; GD - 6 (16.7) 5 (29.4) 1 (5.3) 0.15; HD - 2 (5.6) 0 (0.0) 2 (10.5) 0.39; Smoking, n = 25 (%) 3 (8.3) 2 (11.8) 1 (5.3) 0.68; Pre-pregnancy BMI, kg/m2, median (IQR) 28.8 (10.4%);	no	IGH markers CD3), CD20 and CD68 to visualise the location of respective T-cells, B-cells and macrophages.
Jaiswal N. et al.	27	27	CD 9(33.3) 4(14.8)	Still born 4(14.8) 6(22.2); AS (at 5 min) - 8 11(40.8) 7(25.9); 9 7(25.9) 12((44.5); 10 5(18.5) 2(7.4).	In days, 265.0±18.1 268.48±15.9;	no	Anemia 8(29.6) 7(25.9);	(+)
Kosič N,	1	NP	Maternal vital signs were normal. The postpartum course was unremarkable.	FD, was normally developed, weighing 1190 g and the length of 42 cm.	29 4/7	Controlled HYT and GDM	Uneventful until 28 2/7 weeks	SARS-CoV-2 spike (S) NcP expression placentas
Levitan D. et al.	65	85	PTL 12 (18%), 27 (32%), CD 20 (30%); 36 (42%). 59 (91%) - asymptomatic/ mild/moderate disease, and 6 (9%) had severe/ critical disease. CD (5 of 6 in critical disease). CD - asymptomatic/ mild/moderate SARS-CoV-2 infection (15 of 59).	IUFD occurred in both group	PTL - 12 (18%), 27 (32%),	DM - 2 (3%), 11 (13%). Obesity -34 (52%), 46 (54%)	PE - 9 (14%); 12 (14%).ICP - 5 (8%); P = .01	64 of the placentas IHC staining for SARS-CoV-2 NcP.
Linehan L. et al.	1	5	Emergency CD - distress	(+)	36+4 weeks	Stable HYT	No befor COVID	IGH: CD 68, CD3, CD20, CD138, RTU B-A38), Sars-CoV-2 (Covid-19) Spike Antibody.
Marton T. et al.	1	NP	23-year-old patient with high temperature and reduced fetal movements	Fetal death	25 + 5/40 weeks	Did not have other comorbidities	No befor COVID	CD68 positive histiocytes in the intervillous spaces and the villous trophoblast was positive for the COVID-19 spike protein
Rebutini PZ. et al	19 (10 - asymptomatic, 9 - symptomatic)	19 (birth before the pandemic)	PTL- 10, Term - 9; In the 2-d trimester, 2 woman died due to COVID-19 (1- with her infant 2d trimester)	Infant death - 3 - were positive for SARS-CoV-2 RNA in nasopharyngeal swab or umbilical cord blood samples	33,6 (23–38)	GH - 3, HYT - 4, GD - 4, obesity - 4, bipolar disorder -1, syphilis-1, Hyperthyroidism-1. Situs inversus totalis with metallic stent-1	(+)	To quantify Hofbauer cells (HC), the number of villi and CD68+ cells in those villi were counted in 30 high-power random fields

Table 6. continuation

Şahin O. et al.	30 - with asymptomatic COVID-19	30	CD - 24 (80,0%) vs 15 (50%), p=0,015; NICU - 7 (23.3%) vs 2 (6,7%), p=0,145; uterine atony - 1 (3,3%)vs 0; Maternal nasal O2 requirement 6 (20%) vs 0, p= 0.024	AS <7- 9 (15%), >7 - 51 (85%) vs 2 (6,7%) vs 28 (93,3), p= 0,071;	38+2 weeks	No comorbiduty	GDM - 1 (16,7%); Polyhydramnios 1 (3.3%); PRoM - 1 (3.3%).	NP
Sharps M.C. et al.	28: 8-activ COVID; 20 – post-COVID	16	CD - 4(50%), 11 (55%); control - 13 (81%).	Weight of the infants between groups: 3240 vs 3237 vs 3384 g. 1- recurrent reduced fetal movements; 1- in the control group RFM.	273-274 day	1 - DM, 1 - CH, 1 - HYT,	1- GDM	For the SARS-CoV-2 spike protein, CD163 for macrophages, CD3 for T lymphocytes, CD20 for B lymphocytes or CD31 for endothelial cells.
Smithgall MC,	51	25	CD- 25 (49.0) vs 15 (60.0), VD- 26 (51.0) vs10 (40.0), p= 0.47. Ascending IUI: Maternal response 17 (33.3) vs 9 (36.0), p= 1.00;	IUI: Fetal response 9 (17.7) vs 3 (12.0),p=0.74, no deaths	<37 10 (19.6) vs 4 (16.0), ≥37 41 (80.4) 21 (84.0), p=1,00	Yes - 21 (41.2) vs 12 (48.0), p=0.63; No -30 (58.8) 13 (52.0).	no	IHC with the monoclonal SARS-CoV-2 spike antibody 1A9 for placental evaluation.
Surekha M.V. et al.	122 -seropositive for SARS-CoV-2 IgG, but none reported COVID-19 history	90 (Of the 212, 63.2% (n=134) women were anemic)	Post-dated pregnancy (n=12), cephalopelvic disproportion (n=12), PROM (n=22), , Rh-ve pregnancy (n=8), meconium-stained liquor (MSL) (n=5)	Of 212 case - 3 cases of still births and 7 cases of spontaneous abortions. AS 1 min - 122 (6.20 ± 3.01) 92 (6.21± 3.11) 0.99; AS 5 min - 100 (9.43 ± 0.68) 74 (9.51± 0.58) 0.39	102 (38.22 ± 1.05) vs 78 (38.73 ± 5.82) 0.38	Overweight category (65.5 vs. 76.7%)	134 - anemia, PE (n=3), oligohydramnios (n=51), post-dated pregnancy (n=12), GH (n=2); placenta previa (n=1),	NP
Taglauera E.S. et al.	16	8	Maternal COVID severity – N (%) Hospitalized ICU-N/A 00 vs 1- 3d trim (12.5%)	3353 (459)vs 3453 (327) vs 3446 (411) 0.86; 5-minute APGAR – median (IQR)9 [8.75- 9.00] vs 9 [9- 9]vs 9 [9 - 9], p= 0.12; Required NICU admission – N (%) 1 (12.5%) vs 1 (12.5%) vs 0, p= 1	38.6 (0.9) 39.9 (1.1) 40.0 (1.9) 0.09	Maternal chronic health conditions – N (%)- 5 control (62.5%) vs 3 (37.5%) 2d COVID vs 4 (50%) 3d COVID, p= 0.87	Pregnancy complicationsb – N (%) - 5 (62.5%) vs 7 (87.5%)vs 8 (100%), p= 0.27.	ACE-2 expression, ADAM17 activity and serum ACE-2 abundance in a cohort of matched villous placental and maternal serum samples
Watkins J.C. et al.	7	NP	2 VD, 3 CD; indications spontaneous labor (1 case), PA (1 case).	Fdistress - (2 cases), 1 case - stillbirth. One neonate - complications of prematurity, all remaining neonates were well.	32 weeks - 39+2	Obesity - 1, GH - 1	Abruption -1	Expression of CD4, CD68

Note: ACE – angiotensin converting enzyme, AS – Apgar score, ATH - autoimmune thyroiditis, BMI – body mass index, CA – chorioamnionitis, CD – cesarean delivery, CH – chronic hypertension, CHB - chronic HBV hepatitis, CKD - chronic kidney disease, CNAO - composite neonatal adverse outcomes, COL – cholestasis, CS – case series, FD - foetal distress, FGR - fetal growth restriction, FISH - fluorescence In-Situ Hybridization, GBS – group B- Streptococc, GD – gestational diabetes, GCOL -gestational cholestasis, GDM – gestational diabetes melitus, GH – gestational hypertension, HbC - congenital Hemoglobinopathy, HD - Hypertensive disordersHMD - hyaline membrane disease, HYT - hypothyroidism; ICP – Intrahepatic Cholestasis of Pregnancy, IGH - Immunohistochemical analysis, ISH - in situ hybridization, IUFD – intrauterine fetal death, IUGR – Intrauterine Growth Restriction, IUI – intrauterine infection, NcP - nucleocapsid protein, ND - Neonatal death, NEC – necrotizing enterocolitis, NI – neonatal infection, NICU – Need for intensive care unit, PA – Placental abruption, PE – Preeclampsia, , PPH – postpartum hemorrhage, PPP- post-partum PE, PRoM - Premature rupture of membranes, PTL – preterm labor,RDS – respiratory distress syndrome, SGA – Small for gestational age, TCP- thrombocytopenia, TMPRSS2 - spike transmembrane serine protease 2, VD – Vaginal delivery.

OR = 2.61) and chronic (RR = 8.94, OR = 12.89) inflammatory process in the placenta, as well as the probability of fibrinoid deposition (RR = 9.24, OR = 13.83) and the formation of thrombosis in the intervillous space (RR = 2.94, OR = 3.12) were significantly higher in patients of the main group.

No pathological changes in the placental complex were found in 42±19.62142% of patients in the main group.

Information on perinatal outcomes and the main results of immunohistochemical studies of "covid" placentas is presented in Table 6.

Most of these works reflect a "simple" (conditionally) description of the search for the expression of the SARS-CoV-2 spike protein in placental tissue - 14 (58.33%) studies; 2 (8.33%) papers presented the results of a search for ACE2 receptors, as well as T and B cells of the immune system; 9 works (37.5%) studied the expression of immunocompetent killer cells (CD3, CD4, CD8, CD15, CD19, CD20, CD56, CD68, CD138, CD163. In addition, there are works (5 - 20.83%) that present search data for such cells of the inflammatory process, such as Hofbauer cells, platelets, megakaryocytes; as well as antibodies against alpha smooth muscle cells and cells encoding potassium channels (Kv11) (1 - 4.16%), T and B lymphocytes and macrophages (1 - 4.16%), ADAM-17 (1 – 4.16%)

DISCUSSION

The COVID-19 coronavirus disease pandemic, which began in 2019 in China, affected the entire world and the population of pregnant women involved in the pandemic was no exception, as pregnancy complications and negative perinatal outcomes were not uncommon [47, 48].

Due to the body's aggressive immune response to viral infection in the form of a potentially lethal massive "cytokine storm," COVID-19 is characterized by damage to a wide range of organs and tissues, which leads to various clinical conditions and multiple organ failure [49], incl. endothelial dysfunction and imbalance of pro- and anticoagulation potential of the blood. Within the wide range of pathological manifestations of COVID-19, the high probability of involvement of the utero-placental-fetal complex in the systemic process has attracted special attention. But no clear pathognomic signs were obtained during histopathological evaluation of placentas from women with SARS-CoV-2 viral infection [50].

Based on the results of this systematic review, we conducted a meta-analysis, which allowed us to describe the frequency of histological patterns of the placental complex, taking into account the recommendations of the Amsterdam Placental Workshop Group Consensus Statement (APWGCS, 2016).

MATERNAL VASCULAR MALPERFUSION OF THE PLACENTAL BED IN SARS-COV-2 INFECTION

Common findings most typical of MVM include placental hypoplasia, infarctions, and retroplacental hemorrhage. Microscopic signs include abnormalities in villous development in the form of distal villous hypoplasia or accelerated villous maturation. In most cases, etihistological patterns are not isolated [43]. Our meta-analysis showed that MVM is one of the significantly more frequent events in pregnant women with a "covid history" compared to the control group (38.5% vs7.27%; $F < 0.00001$, $p < .05$).

The information available in the literature regarding the frequency of MVM is extremely conflicting. Thus, according to Tasca S. etal., (2021), the frequency of MVM is only 6%: COVID-19 in the 3rd trimester does not affect the morphology of the placenta and does not cause adverse outcomes for the mother or fetus [22]. Gao L. et al. (2021), despite the presence of signs of MVM in 100% of cases (8 cases with a "covid history" in the 3rd trimester), found no evidence of vertical transmission and adverse outcomes for the mother and fetus [29]. Systematic review on the study of perinatal outcomes in pregnant women with impaired fat metabolism and Sars-CoV-2 infection, which was carried out by Ferraz T. etal. (2023), showed that MVM is observed in 62.85% of women and can lead to negative perinatal outcomes for the fetus. Levitan D. et al. (2021), when ascertaining MVM in the placental complex in 35% and 36% of women, respectively, in the main and control groups, they concluded that direct damage to the placenta is rare, and no pathognomic histological changes specific to COVID-19 infection were found in the placentas [28].

It is known that for most viral infections, overcoming the placental barrier and the development of a generalized inflammatory process with clinical and morphological manifestations is quite characteristic. The main barrier line for vertical transmission of infection is the layer of syncytiotrophoblasts.

The primary receptor of SARS-CoV-2 is angiotensin converting enzyme 2 (ACE2), which ensures the penetration of the virus into the cell and the implementation of its effects. The expression of ACE2 in the cells of the maternal-fetal interface is extremely pronounced: these are syncytiotrophoblast cells, cytotrophoblasts, endothelial cells and smooth muscle cells of the vessels of the primary and secondary villi, but the frequency of vertical transmission of infection, on the contrary, is low. A number of studies have shown the presence of the Sars-CoV-2 virus directly in the syncytiotrophoblast, but the fruits are not infected.

To assess the potential for non-respiratory transmission of SARS-CoV-2 coronavirus infection, Wang W.

et al. (2020), tested samples of bronchoalveolar fluid, sputum, feces, blood and urine of patients for viral RNA and found the following. The frequency of viremia in adults with symptoms of COVID-19 is only 1%, and the SARS-CoV-2 coronavirus is most often detected in fluid bronchoalveolar lavage (93%), sputum (72%), nasal (63%) and pharyngeal swabs (32%). This information highlights the importance and uniqueness of the placental barrier, where maternal and fetal bloodstreams meet and do not mix. between themselves. That is, the probability of infection of the intrauterine fetus by the transplacental hematogenous route is the lowest compared to other routes, which, in our opinion, is a possible explanation for the high frequency of MVM without subsequent infection of the fetus.

FETAL VASCULAR MALPERFUSION IN SARS-COV-2 INFECTION

The main features of FVM include thrombosis, segmental avascular villi, and villous stromal karyorrhexis; Intramural deposition of fibrin in vessels, obliteration of stem vessels, fibromuscular sclerosis and vascular ectasia are also possible. FVM may be segmental due to thrombotic occlusion of chorionic or truncal villous vessels or obliteration of truncal vessels. In global FVM, there is partial obstruction of umbilical blood flow with venous ectasia, intramural fibrin deposition in large vessels, and/or small foci of avascular or karyorectic villi. Obstruction in this case is partial or intermittent, but lesions can spread to most of the placenta up to intrauterine fetal death [43].

According to our review, the frequency of detection of FVM in "covid" placentas ranges from 2.4% (Damman E. et al., 2023) to 78.9% (Rebutini P Z. et al., 2021). If we talk about a comparative analysis of the placentas of a population of healthy women who gave birth (control group) and women who had COVID-19 during pregnancy, FVM was detected in 5.91% (113 cases) vs 28.9% (721 cases) respectively. ($F<0.00001$, $p<.05$).

The results of a number of cohort studies show the following frequency of FVM according to the groups: Glunn S.M. et al. (2022) – 13.2% vs 36.05%, Jaiswal N. et al. (2021) – 7.4% vs 24.67%, Surekha M.V. et al. (2023) – 42.3% vs 55.4%, Sharps M.C. et al. (2022) – 25.5% vs 21.4%. Glunn S. M. notes a higher incidence of fetal vascular lesions in women with symptomatic COVID-19 compared to women with a non-acute form of the disease; according to the authors, this only raises new questions regarding the mechanisms of etiology and clinical consequences of the disease.

According to Rebutini P. Z. et al. (2021), signs of FVM in pregnant women with symptomatic COVID-19 are detected 3 times more often than in women with asymptomatic coronavirus infection (78.9% vs26.31%), but at the same time, compared with the control group of healthy women, morphometric changes there were no pronounced differences in the placenta. Only a significant difference was noted in the frequency of fibrinoid deposition in placental tissues.

Sharps O. et al. (2022) determined the frequency of FVM to be 21.4% and 25.5%, respectively, in the COVID-19 group and the group of healthy postpartum women and concluded that there are no specific Sars-CoV-2-associated changes in the placenta, and the observed increase in the number of placental macrophages may be their response to any possible maternal hypoxia, which increases the release of cytokines in the placental tissue.

Data from our meta-analysis show that FVM is almost 4 times more common in the population of pregnant women with coronavirus infection than in women without a "covid" history, but there is no significant difference between the main and control groups. We emphasize that the histological patterns in the studied placentas are not isolated; in most cases, there is a combination of lesions in both the maternal and fetal bloodstreams.

FVM is a term coined in 2015 by the Amsterdam International Consensus Placenta Pathology Group to characterize a group of non-acute vascular lesions in the placental-fetal circulation [34]. These include various variants of vasculopathies in the fetus of polyetiological origin (parietal and occlusive thrombi, extensive areas of avascular villi, hemorrhagic endovasculitis, thrombosclerosis, fibrinous vasculosis, etc.), which were described in the literature in connection with significant clinical morbidity and mortality of intrauterine fetuses. It is likely to assume that in the conditions of SARS-CoV-2 - associated DIC syndrome, the risk of involvement of the fetal bloodstream in the inflammatory process due to blood stasis, hypercoagulation and endotheliopathy may be a natural process. It is likely that this particular histological pattern of placental changes in pregnant women with a "covid" history may explain the frequency of intrauterine fetal death in these women.

Thus, the high frequency of FVM in its "classical" manifestations in the population of women with coronavirus COVID-19 infection, which our meta-analysis showed, despite the lack of any specificity, is reliable ($F<0.00001$, $P<.05$). However, further research is needed to identify possible specific disorders of coagulation potential and endothelial dysfunction specifically in pregnant women with SARS-CoV-2-associated acute respiratory viral infection.

INFLAMMATORY FINDINGS IN "COVID-19" PLACENTA AS PART OF MATERNAL / FETAL VASCULAR MALPERFUSION

Inflammatory pathological patterns of the placenta or, according to APWGCS, ascending intrauterine infection are of important clinical significance, and its correct documentation is a necessary characteristic of clinical pathology. Histological chorioamnionitis may not be equivalent to clinical chorioamnionitis. The topography and chronicity of the inflammation focus is important, since negative perinatal outcomes are most often associated with the inflammatory reaction of the fetus [43]. Currently, the histological description of acute inflammation in the placenta, extraplacental membranes and umbilical cord is recommended to be designated as acute chorioamnionitis (or acute chorionitis) with or without fetal inflammatory reaction in the chorionic vessels, umbilical vein and/or umbilical artery. Some pathologists may prefer to use the terms "maternal".

In a systematic review that was presented by Hessami K. et al. (2022), it was shown that there are no significant differences in the frequency of maternal (maternal inflammatory response - MIR - OR: 0.66, 95% CI: 0.29–1.52) or fetal inflammatory response - FIR (OR: 0.85, 95% CI: 0.44–1.63), as well as chronic inflammatory lesions (CILs - OR: 0.97, 95% CI: 0.55–1.72) in the placentas of pregnant women with symptomatic or asymptomatic COVID-19.

According to Giordano G. et al. (2022), a report on a series of cases of COVID-19 in pregnant women showed that inflammatory lesions in the form of an acute process (chorioamnionitis) were observed in 20%, and chronic villitis in 100%. It is the chronic aspect of the inflammatory process that is emphasized, but this was not accompanied by vertical transmission of the virus and a significant effect on perinatal outcomes. Horn L.C. et al. (2022), when studying placentas in 2 cases of sudden intrauterine fetal death, revealed a picture of a chronic inflammatory process, despite the asymptomatic course of COVID-19 in these patients. They also noted massive perivillous fibrin deposition and extensive trophoblast necrosis.

Levitan D. et al (2021) reported no differences in the frequency of signs of chronic inflammation in the group of pregnant women with COVID-19 (35%) and the control group (34%); they suggest that there is no direct involvement of the placenta in the systemic process in pregnant women with a "Covid" history [28]. Boyraz B. et al. (2022 – 9%), Damman E. et al. (2023 –– 8.3%), Husen M. F. (2021 – 10.26%) note signs of chronic inflammation in "covid" placentas ranging from 9% to 10.26% and these data did not have significant differences with the control group [30].

There is also contrary information in the literature about the inflammatory component in "Covid" placentas. This is shown in the works of Ferraz T. et al. (2023), Rebutini P. Z. (2021 – 84.21%), Watkins J. C. (2021 – 85.7%), where the frequency of the inflammatory process is more than 80% [25].

According to our review and meta-analysis, signs of acute inflammatory damage to the placenta were found in 7.05% versus 2.82%, and chronic - in 33.22% versus 3.71% of women from the compared groups ($F<0.00001$, $p<.05$).

It should be noted that information about the acute inflammatory process in the placenta, in particular about chorioamnionitis, is extremely contradictory. Thus, the frequency of this pathology ranges from 3.1% (C. Tasca C., 2021) to 63.3% (Surekha M. C. et al., 2023) [22, 47].]. Research by Surekha M. C. (India, CSS) demonstrates a high incidence of acute inflammation in "Covid" placentas compared to a group of anemic pregnant women [37]. The authors note that even oligosymptomatic/asymptomatic maternal COVID-19 infection was associated with more frequent placental damage and this may indicate histopathological features of placental hypoxia and the likelihood of intrauterine fetal hypoxia [47].

We assume that with COVID-19, the placental-fetal complex is involved in the systemic inflammatory process to a greater or lesser extent, is nonspecific in nature, and depends on both the severity of SARS-CoV-2 infection and the comorbid status of the patient.

PERIVILLOUS FIBRIN DEPOSITION IN "COVID-19" PLACENTA AS PART OF FETAL VASCULAR MALPERFUSION

According to the APWGCS (2015) guidelines, fibrin deposition can be subendothelial or intramuscular (intramural) and is part or manifestation of global FVM. These deposits are not occlusive in nature, they are localized in the wall of large fetal vessels, and calcification is possible. The significance of detecting isolated intramural fibrin deposits remains unclear [43]. Small amounts of fibrin are usually detected in normal placenta during physiological pregnancy. The pathohistological picture is characterized by a diffuse increase in the amount of perivillous fibrin, which surrounds the terminal villi and the main stem. The basis of the architectonics of these disorders is the infiltration of placental tissue by maternal immune cells and a decrease in the area between the villous space, which can lead to a deterioration in the perfusion abilities of the placenta and gas exchange with the development of corresponding complications of pregnancy (IUGR,

preterm birth, oligohydramnios, neurological disorders and fetal death ch.).

The pathohistological pattern in the form of intervillous fibrin deposition in "covid" placentas, according to our review, is a significantly common occurrence and occurs in 35.79% of women with a "covid" history versus 3.87% in the control group (F<0.00001, p<.0.5).

According to the literature, the frequency of fibrin deposition in the placentas of women with a "covid" history ranges from 10% (HusenM.F. etal., 2021) to 96.2% (Jaiswal N.et al., 2021). Husen M. F. et al. (2021) identified a specific cluster deposition of CD20+ and B cells around necrotic syncytiotrophoblast in combination with chronic intervillositis and perivillous fibrin deposits in pregnant women with a "covid" history, which was not observed in historical control studies. The authors believe that this localized immunological response in the placental tissue may be due to the presence of SARS-CoV-2 in the placenta and poses a potential danger to the fetus.

There is evidence that the only pathological sign found in "Covid" placentas is an increase in perivillous fibrin deposition -PVFD. Sahin O. et al. (2022), when studying placentas in pregnant women with symptomatic and asymptomatic COVID-19, they determined the frequency of perivillous fibrin deposition as 76.7% vs 56.7%; parietal hypertrophy in placental tissue was found significantly more often in pregnant women with asymptomatic COVID-19 [24].

Watkins J.C. et al. (2021) during a retrospective study of placentas of women with a "Covid" history, incl. and a case of stillbirth, determined that SARS-CoV-2-associated placentitis is characterized by histiocytic intervillositis, perivillous fibrin deposition and trophoblast necrosis. The incidence of perivillous fibrin deposition was 85.7%. The authors believe that these placental features can occur in cases without confirmed transplacental transmission of coronavirus infection, and placental damage is likely mediated by complement activation [25].

INTERVILLOUS THROMBOSIS IN "COVID-19" PLACENTA AS PART OF MATERNAL / FETAL VASCULAR MALPERFUSION

Thrombosis of the intervillous space can be both arterial and venous, and also have different localization, which significantly affects the clinical picture of placental dysfunction [43].

As a result of our review and meta-analysis, we found that the frequency of thrombosis of the intervillous space in "covid" placentas is 6.7% (SahinO., 2022) – 60% (GiordanoG. etal., 2021), while in the group of women without "covid""Anamnesis ranges from 3.3% (SahinO., 2022) to 28% (Smithgall M.C. et al., 2020) [24, 32].

According to a systematic review by Giordano G. et al. (2021), thrombosis is detected in 60% of placental samples, but this pattern is not specific to COVID-19 and does not have a significant impact on perinatal outcomes [66]. Boyraz B. et al. (2022) found that thrombosis is detected in every tenth "covid" placenta (10.4%). There were no placentas with SARS-CoV-2-associated placentitis or evidence of viral infection by immunohistochemistry; the authors suggest that after eliminating the acute inflammatory reaction, persistent infection or chronicity of the process does not occur. However, there has been an increase in the incidence of MVM followed by fetal reaction and FVM with long-term consequences for the fetus. This suggests that the currently existing concept of "long COVID" may also have applied significance to SARS-CoV-2-associated pathology of the placenta.

As for the population frequency of intervillous thrombosis in placentas, according to our data, they are 3 times more likely to accompany a "covid" history in women who have given birth and amount to 8.45% (211 pregnant women) vs 2.87% (55 pregnant women) (F<0.00001, p<. 05).

Taking into account the pathogenetic mechanisms of the development of COVID-19 - the associated syndrome of multiple organ failure and, in particular, disturbances in the coagulation potential of the blood towards hypercoagulation, it is likely and logical to assume that the placenta should become a target for the SARS-CoV-2 virus. In reality, literature data do not support this assumption.

A possible explanation for this fact may be, firstly, the rare involvement of the placenta in the specific SARS-CoV-2 infectious process [28, 29]. Secondly, the placenta is an independent metabolic and hormonal unit of the body, a place of synthesis and implementation the effects of a huge number of enzymes, hormones, biologically active substances. So, for example, a peptidase was found in the placental cyto- and syncytiotrophoblast - aminopeptidase A (L-α-aspartyl-(L-α-glutamyl)-peptide hydrolase, angiotensinase A or glutamyl aminopeptidase, which was isolated biochemically, and its angiotensinase activity is confirmed. This enzyme converts angiotensin II into angiotensin III and its activity increases as pregnancy progresses, which may suggest its participation in reducing the vascular pressor response to angiotensin II in pregnant women. This suggests that angiotensinase A may be a specific counterfactor that promotes the conversion of angiotensin II into the less active metabolite angiotensin III, i.e., thus neutralizing the effect of the promoter of the

SARS-CoV-2 virus - ACE 2. This conclusion is an assumption and requires further research.

Considering that endothelial dysfunction is one of the known mechanisms for the implementation of SARS-CoV-2 - an associated systemic inflammatory response, it seems appropriate to study the interaction of such endothelium-dependent biological messengers of intercellular interaction as prostacyclin, nitric oxide (NO), etc., the synthesis of which is also carried out directly in the placenta; in particular, studies are presented on the role of nitric oxide in the pathogenesis and therapeutic direction of COVID-19.

The correlation between the severity of respiratory viral diseases and NO levels has been known since the H1N1 influenza pandemic, which has led to speculation about the possible effectiveness of NO in COVID-19. Studies have appeared that have confirmed this effectiveness. Possible mechanisms that can neutralize the pathogenic effect of the SARS-CoV-2 virus, incl. in the placenta, there may be vasodilating, direct antiviral and antithrombotic effects of NO.

Thus, we can say that a number of enzymes and biologically active messengers of intercellular interaction are synthesized directly in the placenta, in particular, angiotensinase A and nitric oxide, the activation of which can "strengthen" the placental barrier for the SARS-CoV-2 coronavirus.

OTHER FINDINGS IN "COVID-19" PLACENTA

According to the APWGCS, the concept of "other" pathological changes in the placenta includes an increase in fibrinoid islands with extravillous trophoblast (so-called X-cells), placental pseudocysts, chorionic pseudocysts and membranous decidual necrosis, which cannot be fully characterized as MVM due to the lack of sufficient evidence base [43], but, nevertheless, it is recommended to describe them, which may facilitate further research.

Some researchers have included in this category umbilical cord abnormalities, chorangiosis, hypertrophic arteriolopathy, changes in the basal plate with attached myometrial fibers, microscopic accretism, villous edema, increased circulating nucleated red blood cells or membranes with hemorrhage [28, 29, 32].

According to our meta-analysis, in the population of pregnant women with a "covid" history, the average frequency of detection of "other" placental damage was 17.95% vs 3.66% in the control group ($F<0.00001$, $p<.05$) with fluctuations from 6% (Boyraz B., 2022) up to 78.9% (Rebutini P.Z., 2021). In our opinion, such variability of this indicator emphasizes the lack of specific, pathognomic histological patterns in "Covid" placentas, as well as a unified approach to describing the identified histological patterns - APWGCS recommendations are not always taken into account, which significantly complicates the analysis and interpretation of the results obtained.

CONCLUSIONS

According to currently available studies, in the placentas of women who have had COVID-19 during pregnancy, there are no pathognomic histological patterns specific to this infection and direct damage to the placenta is rarely observed. The probability of infection of the intrauterine fetus by the transplacental hematogenous route is the lowest compared to other routes, which, in our opinion, is a possible explanation for the high frequency of MVM without subsequent infection of the fetus.

Histological patterns in "covid" placentas are not isolated; in most cases there is a combination of lesions in both the maternal and fetal bloodstreams.

The high frequency of FVM in its "classical" manifestations in the population of women with coronavirus COVID-19 infection, despite the lack of any specificity, is reliable. It is likely that the placental-fetal complex in COVID-19 is involved in the systemic inflammatory process to a greater or lesser extent, is nonspecific in nature, and depends on both the severity of SARS-CoV-2 infection and the comorbid status of the patient.

The currently existing concept of "long Covid" may also have applied significance to SARS-CoV-2, an associated pathology of the placenta. It is likely that the activation of a number of enzymes and biologically active messengers of intercellular interaction, in particular, angiotensinase A and nitric oxide, which are synthesized directly in the placenta, can help "strengthen" the placental barrier and prevent the penetration of the SARS-CoV-2 coronavirus.

The variability of various histological patterns in "Covid" placentas emphasizes their lack of specificity, and also reflects the lack of a unified approach to describing the identified histological patterns - APWGCS recommendations are not always taken into account or are not fully taken into account, which significantly complicates the analysis and interpretation of the results obtained.

Further research is needed to identify or deny specific pathohistological changes in the placenta in pregnant women with SARS-CoV-2 associated infection, which may possibly contribute to the development of certain directions for the prevention of pregnancy complications and improvement of perinatal outcomes.

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CONFLICT OF INTEREST
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CORRESPONDING AUTHOR
Gulsym S. Manasova
Odessa National Medical University
6 Marshal Govorov St., 65009 Odesa, Ukraine
e-mail: gulsymmanasova@gmail.com

ORCID AND CONTRIBUTIONSHIP
Gulsym S. Manasova: 0000-0002-1600-5215 A D F
Yana A. Stasy: 0000-0003-2623-860X B
Vyacheslav V. Kaminsky: 0000-0002-5369-5817 E
Igor Z. Gladchuk: 0000-0003-2926-4125 F
Ekaterina A. Nitochko: 0000-0002-3750-0098 C

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ORIGINAL ARTICLE

CONTENTS

Small airways response to bronchodilators as the marker of the uncontrolled asthma in children

Olena Rechkina[1], Svitlana Opimakh[1], Oksana Kravtsova[1], Igor Kaydashev[2]

[1]STATE ORGANIZATION "NATIONAL INSTITUTE OF PHTHISIOLOGY AND PULMONOLOGY NAMED AFTER F.G. YANOVSKY NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE", KYIV, UKRAINE

[2] POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Aim: To develop the criteria of small airways response to bronchodilators (by spirometry indices maximal expiratory flow (MEF50 and MEF25) as the markers of uncontrolled asthma course.

Materials and Methods: The study involved 92 participants (64 boys and 28 girls) aged 6 to 17 years (60 were less than 12 years old) with diagnosed asthma. Asthma control was assessed with the use of Asthma Control Test and Asthma Control Questionnaire. Spirometry and bronchodilator responsiveness testing were performed for all participants.

Results: Mostly, the studied children had a normal level of forced expiratory volume in the first second (FEV1), even at unsatisfactory symptoms control. The indicators of the medium and small airways patency were significantly worse in uncontrolled asthma children even in normal FEV1. Among children, the lack of asthma control can be caused by small airways obstruction in up to 80% cases. Among children who need the high dose inhaled corticosteroids treatment 93.3% have uncontrolled asthma with small airways obstruction. We found out that MEF50 and MEF25 could be the signs of the reversibility of bronchial obstruction and uncontrolled asthma with high sensitivity and specificity.

Conclusions: Indices MEF50 and MEF25 allow detecting the small airways obstruction and their reversibility as a mark of uncontrolled asthma (MEF25 has a higher diagnostic value). In case of MEF50 and/or MEF25 increasing for 22% or 25% accordingly in bronchodilator test in children, the asthma should be considered uncontrolled.

KEY WORDS: children, asthma, spirometry, reversibility, small airway dysfunction, symptoms control

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INTRODUCTION

Bronchial asthma (BA) is one of the most prevalent chronic diseases among children with high global burden. The long term goals for asthma management are good symptoms control to maintain normal activity level and future risks reduction for death, exacerbations, persistent airflow limitation and adverse events. The asthma control evaluation and its monitoring during treatment is very important [1, 2].

Asthma symptoms control assessment in children is subjective and have particularities. For example, the child can avoid physical activity to prevent feel the symptoms and does not understand his physical restriction. Children with chronic airflow limitation often do not feel dyspnea [3]. Separate symptoms evaluation is not sufficient for asthma management, even in mild course of the disease. When symptoms are absent there is a risk of severe exacerbations [1]. In some asthmatic children with good symptoms control persistent lung ventilation impairment and small airway narrowing occur [2]. Moreover, uncontrolled asthma is often a result of small airway involvement [4]. When only symptoms control is taken into account to step down the controller medication, it may lead to severe exacerbations and fixed airflow limitation in future [2].

Control of the future risks include the assessment of the exacerbations, persistent airflow limitation and therapy adverse events. Spirometry is very important tool for future risks assessment in the part of airflow limitation. At the low level of the forced expiratory volume in the first second (FEV1) the probability both exacerbations and fixed bronchial obstruction is increasing. High airway obstruction reversibility is the risk factor for asthma exacerbations. Persistent airway obstruction reversibility in the patients who take maintenance therapy is the sign of uncontrolled asthma. It is recommended for asthmatic children to perform spirometry with bronchodilator test during every visit even with normal FEV1. Positive bron-

Table 1. Demographic and anthropometric characteristics of study population

Characteristics	Mean (SD)	Median (IQR)
Age, years	10.24 (3.3)	9.5 (7.8–12.3)
Height, cm	146 (19)	143 (132–161)
Weight, kg	40.4 (17.20)	35.0 (26.8–52.5)
Body mass index, kg/m^2	18.0 (3.7)	16.8 (15.4–20.3)
Asthma duration, months	43.8 (36.9)	32 (14–60)

Abbreviations: SD – standard deviation, IQR – interquartile range.

chial obstruction reversibility test criteria for children is FEV1 increasing >12% from the start level [1]. One of the functional pattern of the spirometry in asthmatic children is the presence of the distal airway obstruction in normal FEV1 level and positive response to bronchodilator during initially normal FEV1 [3, 5, 6].

FEV1 is the indicator of the air movement through all (large and small) airways. The spirometry indices of theемнгalso should be interpreted. There is maximum mid-expiratory flow (MMEF) that is known as FEF25–75 (the forced expiratory flow at 25–75% of forced vital capacity), which reflects mid/small airways [7]. It was established that FEF25–75 increasing on 18.2% could be interpreted as positive bronchodilator test [8].

The indicators that specified small airway function are maximal expiratory flow (MEF50 and MEF25), the flows where half or 25% of forced vital capacity remains to be exhaled [9]. MEF50 and MEF25 are very important for assessment of the small airway obstruction reversibility, but the literature data how to interpret the MEF50 and MEF25 changes in bronchodilator test in children we have not found.

AIM

The aim of the study was to develop the criteria of the small airways response to bronchodilators (by spirometry indices MEF50 and MEF25) as the markers of the uncontrolled asthma course.

MATERIALS AND METHODS

ETHICS

The study was approved with the local Medical Ethics Committee of the Institute. Participants (or their legal representatives) familiarized with the study protocol and signed an informed consent form to participate in the study.

STUDY POPULATION AND DESIGN

It was observational study in children with asthma, who attended the pediatric department. The study involved 92 participants (64 (69.6%) boys and 28 (30.4%) girls) aged 6 to 17 years with diagnosed BA. Among them 60 were less than 12 years old.

Inclusion criteria: children (male and female) between 6 and 18 years old; diagnosis of asthma is based on the Global Strategy for Asthma Management and Prevention (GINA); asthma duration not less than 6 months; parent(s) or custodian signs the informed consent form before participation; ability to make acceptable spirometry performance.

Exclusion criteria: chronic respiratory illness different from asthma; other clinically significant disease.

We documented demographic, anthropometric data, asthma history, treatment, asthma control and performed spirometry with bronchodilator responsiveness test. Characteristics of patients are listed in table 1.

ASTHMA ASSESSMENT TEST AND QUESTIONNAIRE

Asthma control was assessed with the use of Asthma Control Test (ACT) and Asthma Control Questionnaire (ACQ). The ACT is a specially developed tool for assessing asthma control in children according to age: ACT-child (ACT-C) – for children from 4 to 11 years old, where there are 4 questions for the child and 3 – for parents, and the ACT – for children over 12 years old, questions of which are answered by the child. The level of the ACT and ACT-C less than 20 points indicates uncontrolled asthma (from 5 to 15 points – poorly controlled, from 16 to 19 points – not well-controlled [1, 10]). In ACQ five questions are scoring the symptoms, the sixth question asks about rescue short-acting β2-agonist, the seventh question is the pre-bronchodilator FEV1 percentage predicted. The level of the ACQ equals or more 1.5 points indicates uncontrolled asthma [11].

SPIROMETRY ASSAY

Spirometry was conducted for all participants. Before spirometry, all patients withheld from the use of short-acting bronchodilators (salbutamol – 6 hours, ipratropium bromide – 12 hour), long-acting β2-agonists

Table 2. Clinical characteristics of study population

Asthma treatment:	n (%)	Mean (SD)
ICS	49 (53.5)	
ICS + LABA	16 (17.4)	
ICS + LTRAs	14 (15.2)	
ICS + LABA + LTRAs	7 (7.5)	
LTRAs	6 (6.4)	
ICS dose (for 86 patients who receive ICS):		
Low	33 (38.4)	
Medium	38 (44.2)	
High	15 (17.4)	
ACT for 32 patients over 12 years, score		14.1 (1.6)
ACT < 20	26 (81.3)	
ACT-C for 60 patients 4–11 years, score		17.7 (4.5)
ACT-C < 20	37 (61.7)	
High dose ICS treatment (among 63 uncontrolled patients)	14 (22.2)	
ACQ, score		1.6 (0.7)
ACQ score ≥ 1.5	48 (52.2)	

Abbreviations: SD – standard deviation, ICS – inhaled corticosteroids, LABA – long-acting β2-agonists, LTRAs – leukotriene receptor antagonists.

Table 3. Spirometry results of study patients, mean (SD)

Parameters	Well-controlled (ACT 20 and more points) N=29	Not well-controlled (ACT from 16 to 19 points) N=33	Poorly controlled (ACT from 16 to 19 points) N=30
Pre BD FEV1, % of predicted values	91.8 (19.2)	86.1 (15.7)	85.4 (17.3)
Pre BD FEV1 < 80% of predicted values, n (%)	8 (27.6)	10 (30.3)	11 (33.3)
Post BD FEV1, % of predicted values	97.7 (18.8)	95.8 (16.0)	94.2 (16.5)
Post/Pre FEV1, % change	7.0 (7.8)	12.8 (12.9)*$^{p=0.039}$	11.7 (11.4)
Pre BD MEF75, % of predicted values	78.6 (26.0)	66.2 (20.9)*$^{p=0.048}$	67.5 (23.4)
Pre BD MEF75 < 80% of predicted values, n (%)	15 (51.7)	26 (78.8)	20 (66.7)
Post BD MEF75, % of predicted values	84.5 (27.5)	80.7 (21.5)	84.5 (30.6)
Post/Pre MEF75, % change	10.6 (24.7)	26.4 (30.4)*$^{p=0.031}$	27.1 (33.5)#$^{p=0.038}$
Pre BD MEF50, % of predicted values	74.7 (29.3)	63.6 (21.4)	63.7 (24.5)
Pre BD MEF50 < 80% of predicted values, n (%)	19 (65.5)	26 (78.8)	20 (66.7)
Post BD MEF50, % of predicted values	81.0 (29.1)	80.5 (25.1)	81.1 (24.9)
Post/Pre MEF50, % change	11.1 (22.8)	31.7 (34.1)*$^{p=0.007}$	37.8 (41.6)#$^{p=0.004}$
Pre BD MEF25, % of predicted values	63.8 (29.5)	60.8 (24.4)	59.2 (30,1)
Pre BD MEF25 < 80% of predicted values, n (%)	23 (79.3)	27 (81.8)	27 (70)
Post BD MEF25, % of predicted values	78.4 (34.8)	77.3 (27.5)	73.7 (33.7)
Post/Pre MEF25, % change	28.2 (43.6)	36.5 (50.2)	33.3 (42.1)

Notes. * Differences between well-controlled and not well-controlled groups significant, $p<0.05$.
Differences between well-controlled and poorly controlled groups significant, $p<0.05$.

(formoterol or salmeterol – 24 hours). Patients did not use long-acting muscarinic antagonist. Spirometry was measured using the spirometry device «MasterScreen» 'VIASYS' (Germany). Spirometry tests were performed according standardization of spirometry technical statement [12] and statement on pulmonary function testing in preschool children for six years participants [13]. Children performed spirometry seated in the upright position with the nose clips. Bronchodilator (BD) responsiveness testing performed after acceptable quality pre-dose spirometry. For the children up to 12 years old salbutamol 200 mcg and for the children

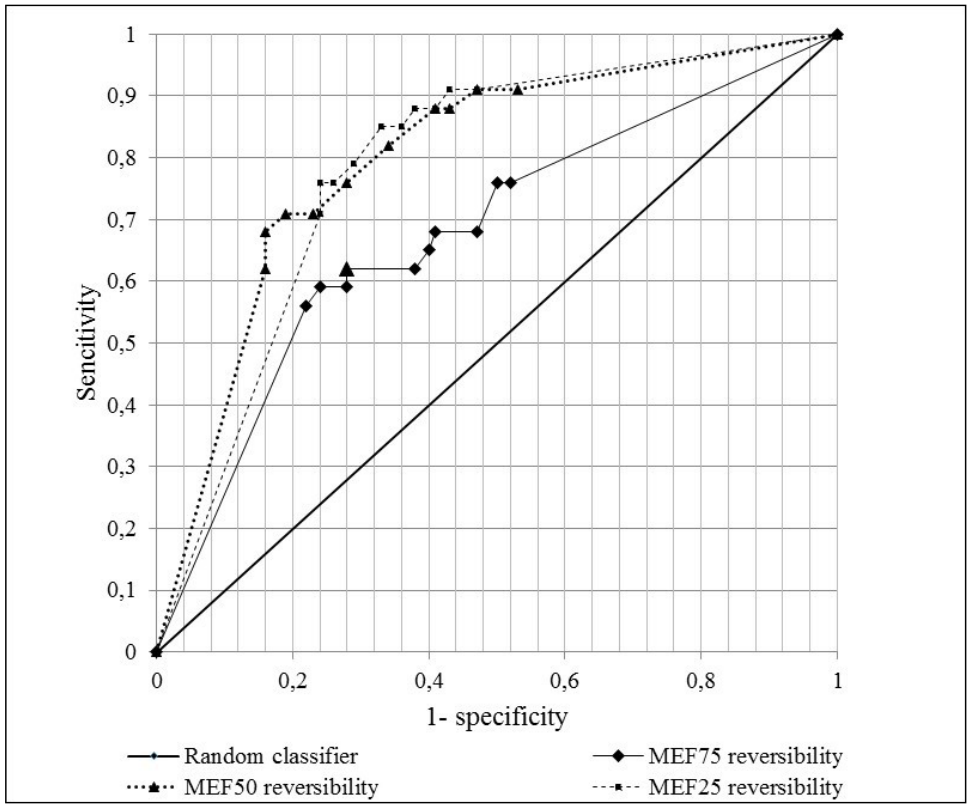

Fig. 1. ROC-curve for MEF75, MEF50 and MEF25 increasing levels as the bronchodilator responsiveness test in asthmatic children.

Table 4. Bronchodilator responsiveness test sensitivity and specificity for MEF75, MEF50, MEF25, %

Reversibility	MEF75		MEF50		MEF25	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
10.0	76.5	48.3	91.2	44.8	91.2	53.4
12.0	76.5	50.0	91.2	46.6	91.2	56.9
14.0	67.6	53.4	91.2	53.4	88.2	58.6
16.0	67.6	58.6	88.2	56.9	88.2	62.1
18.0	64.7	60.3	88.2	58.6	85.3	63.8
20.0	61.8	62.1	82.4	65.5	85.3	67.2
22.0	61.8	72.4	76.5	72.4	79.4	70.7
24.0	58.8	72.4	70.6	76.8	76.5	74.1
25.0	58.8	74.1	70.6	81.0	76.5	75.9
26.0	58.8	75.9	70.6	81.0	76.5	75.9
28.0	58.8	75.9	67.6	84.5	70.6	75.9

Table 5. Characteristics of the diagnostic test to detect the reversibility of bronchial obstruction by the use of MEF50 and MEF25

Parameters	MEF50 %(95% confidence interval)	MEF25 %(95% confidence interval)
Accuracy	73.9 (63.7 – 82.5)	76.1 (66.1 – 84.4)
Sensitivity	76.5 (58.8 – 89.3)	76.5 (58.8 – 89.3)
Specificity	72.4 (59.1 – 83.3)	75.9 (62.8 – 86.1)
Positive predictive value (+PV)	61.9 (50.7 – 72.0)	65.0 (53.2 – 75.3)
Negative predictive value (-PV)	84.0 (73.7 – 90.8)	84.6 (74.7 – 91.1)

after 12 years – salbutamol 400 mcg were used. In 15 minutes after salbutamol inhalation postBD spirometry was performed. Only acceptable measurements were included into analyses. The following spirometry parameters were evaluated: FEV1, MEF75, MEF50, MEF25. All data were presented as percentage of predicted values.

STATISTICAL ANALYSIS

Data collection and statistical analysis were carried out by licensing software products included in the package Microsoft Office Professional using mathematical and statistical functions MS Excel. The studied parameters were evaluated by determining the mean and standard deviation (SD) or median with interquartile range (IQR) for quantitative variables. For the comparison of spirometry results differences in groups with different asthma control, the Student's t test (two-sample, independent samples t-test) was applied. We compared the spirometry parameters means. Verification of numerical series for compliance with the normal distribution was carried out using the special function NORMSAMP_1, developed for the Excel program.

To analyze the characteristics of the diagnostic test, 2×2 tables were built. Accuracy, sensitivity, specificity, positive predictive value (+PV) and negative predictive value (-PV) were calculated [14]. The evaluation of the diagnostic test was conducted with receiver operating characteristic (ROC) curve analysis. The ROC curve charts were built with the use QI Macros add-in for Excel [15]. Area under ROC curve (AUC) was calculated [16].

RESULTS

Asthma treatment and asthma control are listed in table 2. The majority of children received inhaled corticosteroids (ICS) low and medium doses. The most children had uncontrolled course of BA. Among 15 patients who need high doses ICS treatment, 14 (93.3%) had uncontrolled asthma.

We divided patients into three groups by the ACT. There were well-controlled (ACT 20 and more points), not well-controlled (ACT from 16 to 19 points) and poorly controlled (ACT from 5 to 15 points) groups. Spirometry results are presented in table 3.

On average, the studied children had normal level of FEV1, even at the presence of unsatisfactory symptoms control. However, the indicators of the medium and small airways patency significantly worsened in children with uncontrolled asthma even in normal FEV1. The most notable airflow limitation in the middle and small bronchi occurs not in poorly, but in partially controlled group. With symptoms control worsening, more pronounced response to bronchodilator is observed not only for FEV1, but also for medium and small airways. Thus, the indicators of small airway obstruction and their response to bronchodilator can serve as markers of uncontrolled asthma.

During bronchodilator test MEF75, MEF50 and MEF25 increase to significant level not only in patients with an increase in FEV1 by 12.0% or more, but in patients in which FEV1 reversibility varies between 6.0 and 11.9%. It can be assumed that the increase in MEF75, MEF50 and MEF25 during bronchodilator test can confirm the presence of reversible bronchial obstruction in asthmatic children who do not reach the FEV1 increasing by 12.0% or more. In order to define the diagnostic criteria for the bronchial obstruction reversibility, data about the increase in FEV1 (as reference method) and MEF75, MEF50, MEF25 were analyzed. Among the percentage of MEF75, MEF50, MEF25 increasing, the following reference points were analyzed: 10.0; 12.0; 14.0; 16.0; 18.0; 20.0; 22.0; 24.0; 25.0; 26.0 and 28.0% from the level before the inhalation of the bronchodilator as a measure of bronchodilator responsiveness.

It was found that for MEF reversibility level 12.0% after bronchodilator test inherent high sensitivity and low test specificity. At the levels MEF reversibility 14.0, 16.0 and 18.0% after bronchodilator the specificity becomes higher. During test specificity is increasing the sensitivity is falling. If we considered 26.0 or 28.0% as the marker of MEF reversibility, the sensitivity is decreasing without specificity improving (table 4).

To determine the best diagnostic point of MEF75, MEF50, MEF25 increasing in the terms of determining the reversibility of bronchial obstruction in children with asthma, we conducted the ROC analysis. As the ROC curves show (fig. 1), the best point for MEF75 reversibility is 22.0% and more with sensitivity 61.8% and specificity 72.4%. For MEF50 the best point of the bronchodilator reversibility level is also 22.0% with the sensitivity 76.5% and specificity 72.4%. In the case of MEF25, increasing by 25.0% in the bronchodilator test is the marker of bronchial obstruction reversibility with the sensitivity 76.5% and specificity 75.9%.

AUC displays the quality of the diagnostic test while the value 0.9–1.0 means excellent, the value 0.8–0.9 means good, 0.7–0.8 – moderate and 0.6–0.7 means unacceptable quality [16]. In our study AUC for MEF75 reversibility is 0.680, for MEF50 and MEF25 are 0.808 and 0.802 accordingly and such test with the use of MEF75 is less acceptable for clinical practice. Thus, the bronchial obstruction reversibility assessment by MEF50 and MEF25 increasing in bronchodilator responsiveness test has high quality regarding AUC data.

The next step of our study was to analyze the characteristics of the diagnostic test to detect the reversibility of bronchial obstruction by the use of MEF50 and MEF25. To do this, patients were divided into those who had or did not have reversible bronchial obstruction, and 2×2 tables were built. It was found that MEF50 shows the accuracy of the test 73.9%. The prognostic value is high both for the positive result – 61.9% and for negative – 84.0% (table 5). There is no minimum

required sensitivity or specificity for diagnostic tests. However, tests, the sensitivity and specificity of which do not reach 50%, are unacceptable in practice [16]. In our case, the sensitivity and specificity are high – 76.5 and 72.4%, respectively.

When evaluating MEF25, the accuracy of the test was 76.1%, the prognostic value of the test is 65.0% for positive result and 84.6% for negative. Confidence intervals of the calculated characteristics of the diagnostic test confirm its high quality, because in no case the limit of the confidence interval is reduced to below 50%.

DISCUSSION

Isolated symptoms score is not sufficient for the asthma control assessment even with standard questionnaire. Interestingly, in our study the uncontrolled asthma by ACT observed in 63 patients. At the same time among these patients only 48 were uncontrolled by AQC. We explain this as normal FEV1 in the answer for seventh point of the questionnaire reduced the mean value of the poor symptoms.

We focused on the knowledge that obstruction at the level of small airways in children has significant negative effect on the course of BA. It is increasing the frequency and severity of attacks, bronchial hyperresponsiveness to physical exertion and weather factors, which lead to ineffective control of BA, even in patients receiving standard maintenance therapy. Significant bronchodilator responsiveness in patients taking maintain therapy may indicate uncontrolled asthma. Any diagnostic test is useful in conditions of uncertain diagnosis. If it is necessary to detect the reversibility of bronchial obstruction in children, considering the criteria of MEF50 and MEF25 is particularly attractive. On the one hand, it is a part of routine spirometry without involving other diagnostic interventions. On the other hand, MEF50 and MEF25 reflect small airway dysfunction.

The small airways bronchodilator responsiveness in asthma patients has been the subject of interest of various researchers and heterogeneous studies are conducted in this field. As small airways are affected early in obstructive lung diseases, their assessment may be a part of future risk control in asthma management [17].

One of our main findings that in children with uncontrolled BA during spirometry FEV1 is often normal, but MEF75, MEF50 and MEF25 are considerably decreased. This is corresponds with other authors that the reason of the luck of asthma control is small airway dysfunction [18, 19]. The prevalence of small airway dysfunction in asthma patients is 50–60% and observed in all GINA step classes [20]. It is interesting that in the work of Bao W. et al the small airway dysfunction (namely MEF50 and MEF25 decreasing) had such background as violation of the histological structure of the lung tissue despite normal CT imaging and normal FEV1 [21]. However, current literature data regarding small airways dysfunction mainly belong to adults, while we investigate the small airways as the indicator of the uncontrolled asthma course in children from 6 years.

Other our result is establishing relationship between response to bronchodilator of the FEV1 and MEF. The literature data how to interpret the MEF changes in bronchodilator test in children we have not found. We reveal not only FEV1 increasing after bronchodilator is a mark of uncontrolled asthma, but MEF50 and MEF25 post bronchodilator changes indicate this. We were able to calculate the spirometry indices of the reversibility of bronchial obstruction according to the MEF50 and MEF25. When MEF50 increase in the bronchodilator test equal to or greater than 22% and MEF25 increase equal to or greater than 25% it is sensitive in the detection of the losing of asthma control. For MEF50 the accuracy of the test is 73.9%, sensitivity – 76.5%, specificity – 72.4%, area under the ROC curve is 0.808, (for MEF25 – 76.1%, 76.5%, 75.9% and 0.802, respectively), which corresponds to the high quality of the diagnostic test to determine the reversibility of bronchial obstruction. In comparison two parameters: MEF50 and MEF25, the last has the best quality because of higher accuracy, specificity and predictive value.

Thus, evaluation of the clinical and functional status of the patient is important in asthma management. The lack of asthma control in children can be caused by small airways obstruction in up to 80% cases. Among children who need the high dose ICS treatment 93.3% have uncontrolled asthma with small airways obstruction. Further research in larger population-based studies is needed to establish generally accepted parameters for bronchodilator responsiveness testing of small airways to implement them in routine clinical practice.

CONCLUSIONS

1. It is recommended for asthmatic children to perform spirometry with bronchodilator test during every visit even with normal FEV1.
2. When interpreting the results of spirometry in children with bronchial asthma, the level of MEF50 increase in the bronchodilator test equal to or greater than 22% of the value before taking a bronchodilator (or MEF25 increase level equal to or greater than 25%) is proposed as a sign of reversibility of bronchial obstruction.
3. For MEF50 the accuracy of the test is 73.9%, sensitivity - 76.4%, specificity - 72.4%, area under the ROC

curve 0.808, (for MEF25 – 76.1%, 76.5%, 75.9 % and 0.802, respectively), which corresponds to the high quality of the diagnostic test.
4. Spirometry indices MEF50 and MEF25 allow detecting the small airways obstruction and its reversibility is a mark of uncontrolled asthma. Herewith index MEF25 has higher diagnostic value.
5. In case of MEF50 and/or MEF25 increasing for 22% or 25% accordingly in bronchodilator test in children the asthma should be considered uncontrolled.

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CORRESPONDING AUTHOR
Svitlana Opimakh
National Institute of Phthisiology and Pulmonology
named after F.G. Yanovsky National Academy
of Medical Sciences of Ukraine"
10 Mykola Amosova St., 03038 Kyiv, Ukraine
e-mail: sveta_infodoc@ukr.net

ORCID AND CONTRIBUTIONSHIP
Olena Rechkina: 0000-0002-7545-8572 A F
Svitlana Opimakh: 0000-0002-4631-2048 C D
Oksana Kravtsova: 0000-0002-1269-0569 B
Igor Kaydashev: 0000-0002-4708-0859 E

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

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ORIGINAL ARTICLE

CONTENTS

Gut microbiota as an efficacy marker of surgical treatment of obesity

Olexandr Y. Ioffe[1], Pavlo A. Kobzar[1], Oleg V. Shvets[2], Mykola S. Kryvopustov[1], Olexandr P. Stetsenko[1], Tetiana V. Tarasiuk[1], Olena V. Dema[1]

[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

[2]NATIONAL UNIVERSITY OF BIORESOURCES AND NATURE MANAGEMENT OF UKRAINE, KYIV, UKRAINE

ABSTRACT

Aim: To study the impact of bariatric interventions on changes in the parameters of the intestinal microbiome.

Materials and Methods: The research method is a prospective observational cohort monocentric study. 112 patients were included in the study. All patients had indications for surgical obesity treatment due to IFSO criteria. All patients were offered surgical treatment. 53 patients who consented to the operation formed the study group. 59 patients who refused surgical treatment formed the control group. The result of the study was evaluated one year after the start of treatment. The studied group of patients underwent bariatric interventions. The control group consisted of 59 obese patients who were treated conservatively.

Results: evaluating criteria was: %EWL (percentage of excess weight loss), comorbidity regression, life quality improvement. Overwhelming majority of surgically treated patients with gut microbiome composition improvement reached %EWL≥50. Patients who didn't have improvements in gut microbiota composition had insufficient efficacy of surgical treatment.

Conclusions: 1) Surgical treatment of obesity leads to the positive changes in the gut microbiota.

2) Operated patients, who had positive dynamics in changes of gut microbiota demonstrated sufficient efficacy of surgical treatment due to %EWL.

3) Firmicutes/Bacteriodetes ratio and Bacterioidetes/Faecalibacterium ratio can be one of the criteria of the efficacy of surgical treatment of obesity.

4) Patients of the control group, had positive dynamics of changes in gut microbiota much rarely than operated patients and the effectiveness of obesity treatment was insufficient.

KEY WORDS: treatment, gut microbiota, surgical treatment, obesity, %EWL

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INTRODUCTION

The set of microorganisms that colonize the gastrointestinal tract - the intestinal microbiota - its pattern, the ratio of different families of microorganisms is one of the influencing factors in the metabolism of fats and carbohydrates in the intestinal lumen, the degree of their absorption into the bloodstream, and as a result - the development of metabolic syndrome and obesity. According to the literature, the human body is colonized by about 38 trillion microbial organisms, of which about 90% colonize the gastrointestinal tract [1]. More than 35,000 bacterial families have been classified in the colon, among which *Firmicutes* (which includes Gram-positive species), *Bacteroides* (which includes Gram-negative species), *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* are the most common. The indicated families, groups and species represent approximately 90% of the entire microbiome of the large intestine. The most widespread representatives of the microbiota are obligate anaerobes from the species *Bacteroides, Eubacterium, Clostridium, Ruminococcus, Peptococcus, Peptostreptococcus, Bifidobacterium,* and *Fusobacterium* and facultative anaerobes such as *Escherichia, Enterobacter, Enterococcus, Klebsiella, Lactobacillus*, and *Proteus* [2]. *Firmicutes*/*Bacteriodetes* ratio and *Bacterioidetes*/*Faecalibacterium* ratio are indicators that represent the ratio of the predominant number of microorganisms that are present in the colon. It was established that these ratios are an indicator of the physiological status of the intestinal microbiome, and change in response to changes in eating behavior and the occurrence of methabolic disorders and obesity in the studied individuals. However, the relationship between the changes in the intestinal microbiota and the surgical treatment of obesity and its effectiveness is still being studied, so the analyzis of the changes in the intestinal microbiome after bariatric surgery is extremely relevant [3].

Bariatric surgery is increasingly used in most countries of the world as an effective and safe method of obesity

treatment. According to the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) 8th Global registry report, in 2023, 480,970 bariatric operations were performed in the world. The most common metabolic interventions are sleeve gastrectomy - 290,505 operations (60.4%), Roux-en-Y gastric bypass - 141,886 operations (29.5%), mini gastric bypass - 20,681 operations (4.3%) and other surgeries in the amount of 27,896 (5.8%) such as Single anastomosis duodenoileal bypass (SADI), biliopancreatic diversion, gastric banding and endoscopic installation of an intragastric balloon. Among these operations, 93.47% were performed initially, and 4.11% were provided as revision interventions. Bariatric interventions are performed more in obese women: 82.2% of all interventions than in men - 17.8% [4].

In some scientific publications, changes in the intestinal microbiota were evaluated depending on the body weight of the subjects, and changes in the intestinal microbiota after bariatric interventions [5], but the effectiveness of surgical treatment of obesity depending on the changes in the intestinal microbiota in response to the performed bariatric intervention has not yet been investigated. Therefore, the aim of the study was to study the effectiveness of surgical treatment of obesity due to changes in the intestinal microbiota.

AIM

The purpose of the study was to study the impact of bariatric interventions on changes in the parameters of the intestinal microbiota.

MATERIALS AND METHODS

The research method is a prospective observational cohort monocentric study, which was conducted on the basis of the Department of General Surgery No. 2 of the O.O. Bogomolets National Medical University.

112 patients were included in the study (clinical characteristics of the patients are presented in Table 1). All patients had indications for surgical treatment due to IFSO criteria, namely BMI ≥ 35 kg/m2 regardless of the presence and severity of comorbid diseases, patients with BMI 30-34.99 kg/m2 and existing comorbid conditions [6]. All patients included in the study were offered surgical treatment. 53 patients who consented to the bariatric procedure were included in the study group. 59 patients who refused surgical treatment and underwent conservative treatment formed the control group. The result of the study was evaluated one year after the start of treatment. The studied group of patients underwent the following bariatric interventions: laparoscopic gastric bypass – 20 patients (37.7%), laparoscopic sleeve gastrectomy – 17 patients (32.2%), and 16 patients (30.1%) with superobesity (BMI≥50 kg\m^2) who underwent two-stage surgical treatment [7]: the installation of an intragastric balloon for a period of 6 months as the first stage of the two-stage treatment, followed by gastric bypass within 14 days after the removal of the balloon and the assessment of the final result 12 months after the start of the two-stage treatment [8]. The control group consisted of 59 obese patients who were treated conservatively: individual diet prescription, psychological support sessions, lifestyle correction, and dosed physical activity.

In both groups, at the beginning of the treatment and 12 months after the treatment, the following studies were performed - anthropometric examinations, routine general clinical examinations and stool analysis by the PCR method to determine the quantitative and qualitative indicators of the intestinal microbiome.

The following criteria were used to assess the effectiveness of treatment.

%EWL – percentage of excess body weight loss. The American Society for Metabolic and Bariatric Surgery considers a %EWL (Excess Weight Loss) of at least 50% in the 12-month postoperative period as an indicator of effective surgical treatment of obesity [9].

Positive changes (positive changes are taken to be the return of the specified microbial ratios to the reference values) of the intestinal microbiota [10]. The main studied parameters of changes in the intestinal microbiota are *Firmicutes*/*Bacteriodetes* ratio and *Bacterioidetes*/*Faecalibacterium* ratio - the ratio of the predominant number of representatives of the families of microorganisms that colonize the intestinal mucosa. Average European normal indicators of the indicated colonies are 1-5 for *Firmicutes*/*Bacteriodetes* ratio and 0.01-100 for *Bacterioidetes*/*Faecalibacterium* [11, 12].

Regression of comorbid conditions was considered a return to the reference values of indicators of carbohydrate metabolism (blood glucose level, glycosylated hemoglobin), fat metabolism (LDL, LDL), blood pressure indicators and cardiac function parameters [13].

RESULTS

The average BMI of patients in the study group before surgery was 48.6 ± 20.1 kg/m2, among patients in the control group the average BMI before treatment was 41.6 ± 16.0 kg/m2. After the treatment, the average BMI of the studied BMI decreased to the level of 29.5 ± 5.4 kg/m2, in patients of the control group it was 40.2 ± 13.8 kg/m2 (Table 2).

In the patients of the study group, the average %EWL after 12 months of observation was 59.21% ± 23%. The

Table 1. Clinical characteristics of obese patients included in the study

	All patients included in the study, n=112	Study group, n=53	Control group, n=59	p*
Age, years	48,2±9,2 (27 - 68)	47,7±9,1 (23 - 68)	48,9±9,5 (29 - 67)	0,286**
Body mass, kg	144,8±15,7 (110,14 – 210,22)	148,03±18,38 (110,14 - 210,22)	141,8±12,2 (117,2-172,2)	0,085**
Height, sm	165,62 ± 12,6 (150 – 188)	165,27±11,7 (152 - 188)	165,91±13,3 (150 -185)	0,818
Initial BMI, kg/m^2	45,1±6,1 (35,2 - 75,3)	48,6±7,4 (35,2 - 75,3)	41,6±3,1 (35,5 - 50,5)	0,256**
Ideal body mass, kg	62,2±7,2 (51,5 – 74,5)	62,6±7,1 (52,8 – 73,3)	61,2±6,8 (51,1 – 74,5)	0,362**
Excess weight, kg	100,2 ± 19,0 (65,9 - 144,6)	101,5 ± 22,3 (70,5 - 144,6)	99,8 ± 18,5 (62,3 - 132,7)	0,321**

* - comparison of data in the studied and control groups

** - in one or both groups, the distribution of data was different from normal, the analysis was performed using the Wilcoxon T-criteria.

Table 2. BMI dynamics in patients of both groups depending on the type of treatment

Type of treatment	BMI, kg/m^2		p*
	Before treatment	After treatment	
Gastric bypass	47,8 ± 10,1 (35,2 – 75,3)	28,5±6,2 (26,2 – 30,1)	<0,001
Sleeve gastrectomy	42,2±7,4 (35,4 – 48,6)	30,1±5,5 (29,2 – 31,0)	<0,001
Intragastric balloon+gastric bypass**	52,2±6,4 (50,1 – 54,4)	28,3±6,0 (26,1 – 30,5)	<0,001
Average in study group	48,6 ± 20,1 (35,2 – 75,3)	29,5 ± 5,4 (26,1 – 31,0)	<0,001
Conservative tratment	43,6 ± 16,2 (35,5 – 50,5)	40,2 ± 13,8 (33,4 – 47,0)	0,06

*comparison was made between patients of the study and control groups;

**intragastric baloon+gastric bypass – installation of an intragastric balloon as the first stage of treatment of superobese patients and performing gastric bypass 6 months after the start of treatment [7].

highest rate of %EWL of patients in the study group was 77.2%, the lowest was 49.9%. In patients of the control group, the average %EWL was at a significantly lower level – 9.92% ± 7.25%. The interval between the highest and lowest %EWL in the control group is 19.45% and 2.2%, respectively.

The average %EWL in patients who underwent bariatric surgery varies depending on different surgical techniques. Patients who underwent gastric bypass method showed the highest %EWL – 69.71% ± 20.0%, patients who underwent sleeve gastrectomy had an %EWL – 51.57% ± 5.9%, and patients after two-stage surgical treatment - average %EWL 51.7% ± 7.92%.

During the analysis of the quantitative changes in the gut microbiota composition, significant differences were found in response to the treatment in both groups. Thus, when analyzing the *Firmicutes*/*Bacteriodetes* ratio (FBR) and *Bacterioidetes*/*Faecalibacterium* ratio (BFaR) in the studied group, it was established that the average FBR before surgical treatment was 94.5. FBR before treatment was within the reference values only in three patients of this group (5.67%). The average BFaR in the studied group was 1708.5 (there is a significant range of minimum and maximum indicators in the group), 50000 and 0.0003, respectively. BFaR before surgical treatment was within the reference values in two patients (3.77% of the group).

During the analysis of the changes that occurred in the intestinal microbiota patterns of the patients, 12 months after the surgery, the following changes were found. The average FBR after the surgical treatment of obesity was 2.84 ± 6.4, the interval of the maximum and minimum values was 7.19 and 0.79, respectively. Gut microbiota of 50 patients (94.33% of the group) reached reference values. Three patients (5.67%), who did not have positive changes in FBR indicators, had insufficient effectiveness of surgical treatment in terms of % EWL - an average of 49.52%±0.6. The average BFaR

Table 3. Gut microbiota changes in relation with the type of treatment.

Treatment type	Firmicutes/Bacteriodetes ratio		p*	Bacterioidetes/Faecalibacterium ratio		p*
	Before treatment, % of referent values	After treatment, % of referent values		Before treatment, % of referent values	After treatment, % of referent values	
Gastric bypass	5,00	95,00	<0,001 #	10,00	95,00	<0,001 #
Sleeve gastrectomy	0,00	94,22	<0,001 #	5,82	94,28	<0,001 #
Intragastric balloon+gastric bypass **	6,25	87,50	<0,001 #	0,00	81,25	<0,001 #
Study group (average)	5,67	94,33	<0,001 #	3,77	92,40	<0,001 #
Control group	3,38	16,90	0,06	0	18,60	0,07 #

in one or both groups, the distribution of data differs from normal, the comparison was carried out using the Wilcoxon T-criteria;
* comparison was made between patients of the study and control groups;
**intragastric baloon+gastric bypass – installation of an intragastric balloon as the first stage of treatment for superobese patients and performing gastric bypass 6 months after the start of treatment [7].

in operated patients after 12 months was 58.4, with a range of the highest and lowest indicators – 121 and 0.256, respectively. In 49 patients (92.4% of the group), microbiota reached reference values. Four patients (7.6%) did not have intestinal microbiota indicators return to reference values and had insufficient effectiveness of surgical treatment in % EWL - the average indicator was 49.33%±1.1.

In patients of the control group, the intestinal microbiota changed in response to the start of treatment in a different way. The mean FBR before treatment was 52.5 ± 91.1. In two patients of this group (3.38%), the average FBR fluctuated within the reference values before the start of treatment. The mean BFaR before treatment in the control group was 579.1. BFaR reference values before treatment were not found in the control group.

After treatment in the control group, the average FBR was 6.1 ± 22.2 (range 0.2 - 22.4). Ten patients of the control group (16.9%) demonstrated results within the reference values from the start of treatment. In turn, the average BFaR in patients who underwent a course of treatment were 130.2; this parameter reached reference values in eleven patients (18.6%) (Table 3).

In the course of the statistical analysis of the obtained data, the linear correlation method confirmed the existence of a strong positive correlation between the surgical treatment of obesity and the effectiveness of the treatment according to %EWL, r=0.969 at p≥0.001, and the absence of a correlation between the choice of conservative methods of obesity treatment and treatment efficiency according to %EWL, r=0.172 at p≥0.01. A strong negative rank correlation was also found between the value of BMI (degree of obesity) and the frequency of detection of reference indicators of intestinal microbiota in the sample, r=0.798 at p≥0.05. Only 2.67% of patients had a pattern of intestinal microbiota close to the reference values before the start of treatment. In turn, in the postoperative period of patients in the study group, in 93.3% of cases there were changes in the patterns of the intestinal microbiome and their return to the reference values, which correlates with the degree of reduction in BMI r=0.717 (p≥0.05), and %EWL r=0.633 (p≥0.001). There is a correlation between the choice of surgical method of obesity treatment and the return to normal FBR and BFaR indicators r=0.628 and r=0.642 (p≥0.05). During the analysis of the data of patients of the control group, no relation was found between the effectiveness of the treatment according to %EWL and the normalization of intestinal microbiota indicators r=0.077 (p≥0.05) of the control group 17.7% of patients of the control group had positive changes in the patterns of intestinal microbiota , however, the correlation with the effectiveness of the treatment was not found r=0.112 (p≥0.05).

DISCUSSION

During the research, a number of regularities and tendentions were revealed.

Intestinal microbiota is a variable functional unit of intestinal work and metabolism as a whole. Changes in the composition of intestinal microbiota occur in response to changes in diet and eating behavior [1-3]. Christopher L. Gentile, Tiffany L. Weir and others. in

their study of the functioning of the intestinal microbiota, they found that a change in the nature of the diet, especially an increase in the amount of fat, causes changes in the composition of the intestinal microbiota and a number of metabolic disorders, even before the appearance of overweight and obesity [1]. Gomes AC, Hoffmann C, et al. in their study indicate that changes in the pattern of intestinal microbiota mediated by a diet with a high content of fats and carbohydrates cause a pro-inflammatory response in the lymphoid tissue of the large intestine, which leads to a violation of the mechanisms of nutrient absorption, a violation of the hunger-satiety regulation system, and leads to changes in eating behavior, namely overeating [3].

Bariatric surgery is also an important factor affecting changes in intestinal microbiota patterns. The operated patients included in the study in the vast majority of cases demonstrated positive dynamics of changes in the composition of the intestinal microbiota in the postoperative period, in comparison with indicators of functioning of the microbiota before surgical treatment of obesity. According to the study of Gutiérrez-Repiso, C., Moreno-Indias et al. intestinal microbiota after sleeve gastrectomy and gastric bypass according to the Roux method, changes during the study period and in comparison with the preoperative pattern of intestinal microbiota. In their study, the authors are looking for the possibility of identifying a specific composition of the intestinal microbiota that could predict remission of type 2 diabetes or unsuccessful weight loss after bariatric surgery [5].

Bariatric interventions are more effective than conservative methods and have a greater impact on the intestinal microbiota. Silvia Palmisano, Giuseppina Campisciano and others. in their study evaluated the relationship between gastric bypass and changes in the intestinal microbiota and concluded that bariatric interventions have a greater clinical effect in cases accompanied by positive dynamics in the composition of the intestinal microbiota, compared to patients who did not have the indicated changes [14] .

Changes in the composition of the intestinal microbiota after surgical treatment of obesity are interrelated and play a role both in the functioning of the intestine after the intervention and in the metabolism of nutrients, so they can be attributed to the factors affecting the effectiveness of surgical treatment.

CONCLUSIONS

1) Effective surgical treatment of obesity leads to the positive changes in the intestinal microbiota in 93.3% of cases ($p \leq 0.001$), in contrast to the control group, where microbiota approached the reference values only in 17.7% of cases ($p \geq 0.06$).
2) Patients of the studied group, who had positive dynamics in changes of gut microbiota in 98.1% of cases, had the effectiveness of obesity treatment due to %EWL at the level of 59.21% ($r=0.717$; $p \leq 0.001$). %EWL ≤ 50% was observed in patients who did not have positive changes in intestinal microbiota after surgical treatment.
3) *Firmicutes*/*Bacteriodetes* ratio and *Bacterioidetes*/*Faecalibacterium* ratio can be one of the criteria for evaluating the efficacy of surgical treatment of obesity.
4) In patients of the control group, positive dynamics of changes in gut microbiota composition occurred in 17.7% of cases and the average effectiveness of obesity treatment by %EWL was 9.92% ($r=0.077$; $p=0.06$).

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CONFLICT OF INTEREST

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CORRESPONDING AUTHOR

Pavlo A. Kobzar
Bogomolets National Medical University
13 Taras Shevchenko Boulevard, 01601 Kyiv, Ukraine
e-mail: kopasha1996@gmail.com

ORCID AND CONTRIBUTIONSHIP

Olexandr Y. Ioffe: 0000-0002-1306-7920 A F
Pavlo A. Kobzar: 0000-0003-2802-674X A B C D
Oleg V. Shvets: 0000-0002-1434-4344 A
Mykola S. Kryvopustov: 0000-0003-4978-4873 C
Olexandr P. Stetsenko: 0000-0002-2219-653X E
Tetiana V. Tarasiuk: 0000-0001-6629-3908 E
Olena V. Dema: 0000-0002-7451-3816 E

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

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CONTENTS

Association between rs1799983 polymorphism of *eNOS* gene and essential hypertension in Iraqi hypertensive patients

Ghufran K. Salman, Bassim I. Mohammad, Hussein A. Saheb, Ahmed M. Sultan, Sinaa Abdul Amir Kadhim, Asma A. Swadi

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, COLLEGE OF MEDICINE, UNIVERSITY OF AL-QADISIYAH, IRAQ

ABSTRACT

Aim: To investigate allele frequencies of rs1799983 polymorphism *eNOS* genes and to determine association between rs1799983 polymorphism of *eNOS* gene and essential hypertension in Iraqi hypertensive patients.

Materials and Methods: This is an observational cross sectional descriptive single center study. ninety hypertensive patients were recruited by specialist cardiologist and conducted at AL-Diwaniyah teaching hospital and department of pharmacology and therapeutics, college of medicine, university of Al-Qadisiyah, Iraq. DNA samples were genotyped by PCR-tetra-arm method. NO level was measured by using ELISA kit.

Results: Regarding rs1799983 the most frequent allele was G (73%) and the most frequent genotype was GG (55%). Our results indicate lack of substantial link between genotype frequencies of rs1799983 polymorphism and NO level (p=0.88) and thereby there is no statistically significant effect on SBP and DBP (p = 0.051).

Conclusions: our study demonstrated lack of significant association between this polymorphism and essential hypertension in Iraqi hypertensive patients.

KEY WORDS: rs1799983 polymorphism, *eNOS* gene, essential hypertension, Iraqi hypertensive patients

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INTRODUCTION

Due to the fact that the majority of individuals with hypertension (HTN), a prevalent chronic treatable condition, are asymptomatic, it is sometimes known as "the silent killer". Despite advancements, doctors still encounter difficulties in achieving optimal blood pressure (BP) values in all hypertensive patients because of these patients' variable medication responses. BP is the result of peripheral vascular resistance (PVR) and cardiac output (CO) [1]. Therefore, it is believed that elevated CO and/or elevated PVR are the causes of hypertension. Heart rate (HR) and stroke volume together determine CO; the size of the vascular compartment and myocardial contractility both affect stroke volume. Nearly half of the adult population in the US has hypertension, according to the American Heart Association's 2017 recommendations [2, 3]. In Arab nations, it is thought that 30% of adults suffer from hypertension [4]. Follow-up research conducted in Jordan examining the prevalence of hypertension between 1994 and 2009 found that it increased from 29.4% to 32.3% [5]. According to data on the prevalence of hypertension from 2009 to 2017, about one-third of Jordanian adults had the disease [6]. In 2008 the prevalence of HTN in Iraq for both sexes was 29.4 according to the World Health Organization (WHO) Eastern Mediterranean Region health statistics [7]. The prevalence of HTN in Thi-Qar was 26.5% in 2014 [8]. Hypertension is a multifactorial disease, and the causes for these disappointing results are complex, including drug non-adherence, which may be due to treatment costs or adverse effects, and inter-individual genetic variability [3]. Due to the polygenic character of HTN, it is particularly challenging to identify the single nucleotide polymorphisms most likely to be linked with essential hypertension and also the connections between certain genes and treatment responses in various ethnic groups [4]. One of important genes is *NOS3* gene, which encode eNOS enzyme that mediate nitric oxide (NO) production. Nitric oxide is a vasodilator substance that produced by endothelium. Endothelial dysfunction may be related to both direct pressure-induced damage and increased oxidative stress in the presence of chronic HTN (oxidative stress is caused by either decreased breakdown or increased generation of reactive oxygen species (ROS)). By producing too many free radicals, such as superoxide anions, which bind to NO, ROS can exacerbate vascular dysfunction by increasing the production of the proinflammatory

oxidant ($ONOO^-$) and reducing nitric oxide bioavailability. Reduced nitric oxide bioavailability is a crucial factor linking oxidative stress to hypertension and endothelial dysfunction [9]. Under normal circumstances, NOS catalyzes the conversion of electrons from NADPH, arginine and oxygen into citrulline and NO. The co-factors tetrahydrobiopterin (BH4), oxygen and NADPH are among those known to be necessary for NOS [2, 3]. After being synthesized, it is transferred from the endothelial cell membrane to vascular smooth muscle cells, where it activates guanylate cyclase and causes it to convert GTP to cGMP, which subsequently leads to calcium removal and cell relaxation [10]. Elevated blood pressure and the development of HTN are brought on by the interruption of NO synthesis via suppression of constitutively expressed eNOS in both animal and human [11]. The *NOS3* or *eNOS* gene encodes the endothelial nitric oxide synthase (eNOS). The *eNOS* gene is found on chromosome 7q36 and is made up of 26 exons and 25 introns that encode for a one hundred-thirty five KDa proteins with one thousand- two hundred three amino acids that span approximately 23 kilobases of the genome (Fig.1). *eNOS* gene is highly polymorphic [12, 13].

AIM

The main aim of the present research is to investigate allele frequencies of rs1799983 polymorphism eNOS genes and to determine association between rs1799983 polymorphism of eNOS gene and essential hypertension in Iraqi hypertensive patients.

MATERIALS AND METHODS

This is an observational cross sectional descriptive single center study for hypertensive patients of Iraqi nationality, diagnosed according to JNC-8. All candidate patients diagnosed and recruited by specialist caregiving physician/cardiologist. The study was conducted at Al-Diwaniyah teaching hospital and Department of Pharmacology and Therapeutics, Medicine College, Al-Qadisiyah University, Iraq.

SUBJECTS

Ninety adults (37 male and 53 female) aged 20-70 years old were enrolled in this study. Patients with renal or hepatic impairment, pregnancy, heart failure, obesity (BMI ≥ 30) and psychiatric patient were considered as exclusion criteria. The study was approved by the Ethics Committee of the Medicine College, University of Al-Qadisiyah and procedures were explained to all participants and informed consent was taken from all patients.

BLOOD SAMPLE

Blood samples of 4 ml were collected from the patients that were aspirated from antecubital vein divided into two portions. One milliliter (ml) of the patient's whole blood were collected in a tube containing EDTA for DNA extraction and stored at -20 C until the time of DNA extraction. Three milliliters (ml) of the patient's whole blood collected in a gel tube, spun at 5,000 revolutions per minute for five minutes, and the serum was collected to be used in biochemical tests.

DNA ISOLATION AND GENOTYPING

Genomic DNA from blood samples was isolated using a DNA extraction kit (Frozen Blood) (Geneaid, USA). The tetra-primer ARMS-PCR technique was performed for genotyping and detecting *eNOS* (rs1799983) gene polymorphism in blood samples. In order to identify the genotype, the ARMS-PCR tetra-primer uses 4 primers in a single PCR. Two non-allele specific primers amplify the area containing the single nucleotide polymorphism at the beginning of the reaction. Therefore they are called outer primers. Two allele-specific primers, called inner primers, which will produce allele-specific fragments, use the outer fragment of the primer as a template in its production [14]. Two allele-specific fragments in an agarose gel can be recognized by their differing diameters by positioning the outer primers at various distances from the polymorphic nucleotide [15]. The online website "PRIMER1: primer design for tetra-primer ARMS-PCR" (http://primer1.soton.ac.uk/primer1.html) was used for design primers (Table 1). BLAST program in NCBI server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to test the primers specificity.

PCR conditions were as following: after denaturation at 95°C for 5 min, 35 cycles were performed (95°C for 1 min, annealing temperature 63°C for 1 min, followed by extension at 72°C for 1 min) and final extension at 72°C for 7 min to amplify the target DNA. DNA were separated by electrophoresis on 1% agarose gel and visualized with ethidium bromide. (Fig.2).

STATISTICAL ANALYSES

Statistical analyses were made by SPSS version 25. For each SNP, allele and genotyping frequencies were calculated. P value <0.05 was considered statistically significant. The data were shown as mean ± SE.

RESULTS

The genotype frequencies of the rs1799983 (G894T) polymorphism among patients with essential hypertension did not significantly differ from those predicted un-

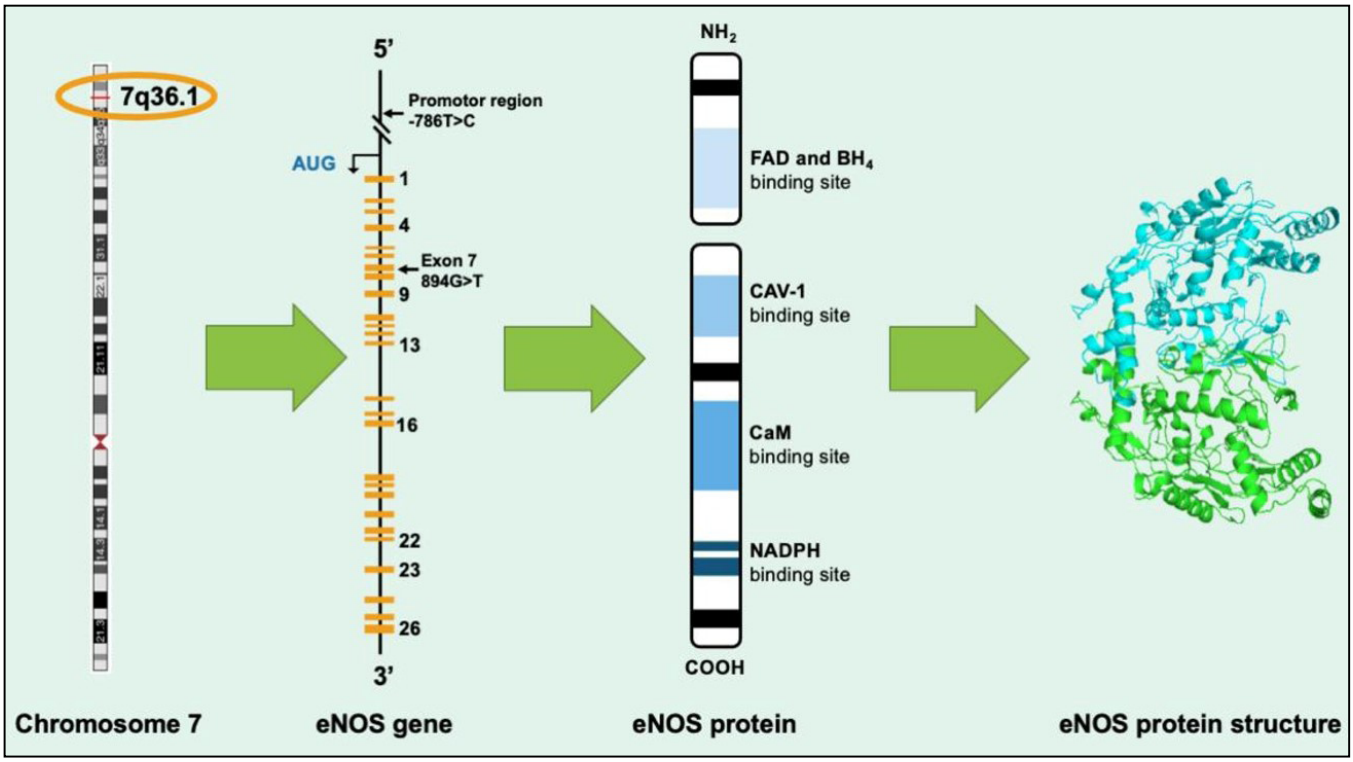

Fig. 1. Location of the endothelial nitric oxide synthase gene (eNOS) on chromosome 7.

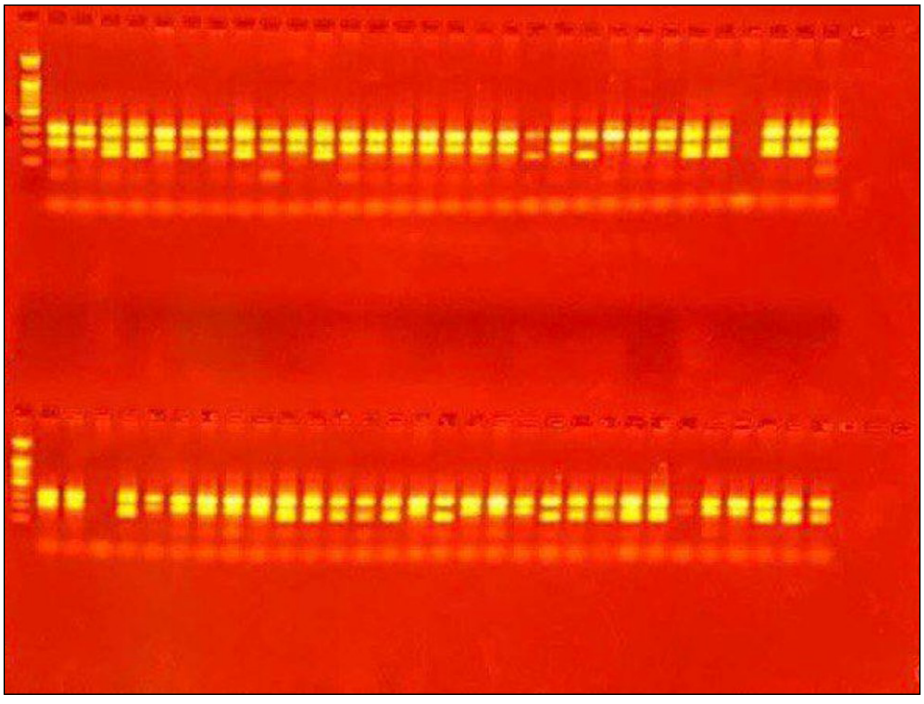

Fig. 2. Agarose gel electrophoresis image that show the PCR product analysis of eNOS (rs1799983) gene from some blood sample patients.

der conditions Hardy- Weinberg equilibrium (P>0.05). The genotype frequencies (GG, GT, TT) were 48 (55%), 31 (36%) and 3 (9%), respectively, with the most common allele being G - 127 (73%). The mean ± SE plasma level of NO in homozygous GG carriers was 72.3±2.9 µmol/L. In homozygous TT carriers, the plasma level was 74.6±9.4 µmol/L. On the other hand, plasma level of NO in heterozygous GT was 74.7±3.9 µmol/L. There was no statistically significant association between genotype frequencies and NO level (p = 0.88) (Table 2).

As shown in Table 3, the mean ± SE systolic blood pressure in homozygous GG, heterozygous GT, and homozygous TT carrier patients was 149 ± 14.5 mmHg, 152 ± 15.4 mmHg, 150 ± 15.9 mmHg respectively. On other hand, the mean ± SE diastolic blood pressure in homozygous GG, heterozygous GT and homozygous TT carrier patients was 87.9 ± 6.6 mmHg, 91.6 ± 6.7 mmHg, 87.8 ± 6.8 mmHg respectively. There was no statistically

Table 1. The PCR primers with their sequence, amplicon size and annealing temp

Primer	Sequence	Amplicon	Annealing
eNOS rs1799983	Inner forward CTGCTGCAGGCCCCAGATAAT	T-allele 147 bp.	63 ℃
	Inner reverse ACCCTGGAGATGAAGGCAGGA	G-allele 195 bp.	
	Outer forward GCAGAAGGAAGAGTTCTGGGAGC	Two outer primers 298 bp.	
	Outer reverse CCTTCTTGAGAGGCTCAGGGATG		

Table 2. Mean ± SE values of NO level and genotyping frequency in Iraqi patients with essential hypertension eNOS rs1799983 (G894T) polymorphism

Genotype rs1799983 (G894T)	Numbers	Mean NO level (µmol/L)	S.E	P value
GG	48	72.3	2.9	0.88
GT	31	74.7	3.9	
TT	8	74.6	9.4	

Table 3. Effect of eNOS rs1799983 (G894T) polymorphism on systolic and diastolic blood pressure in Iraqi hypertensive patients

Genotype rs1799983	Systolic BP mean (mmHg)	S.E	P value	Diastolic BP mean (mmHg)	S.E	P value
GG	149	2.1	0.6	87.9	0.9	0.051
GT	152	2.7		91.6	1.2	
TT	150	5.6		87.8	2.4	

significant effect of eNOS rs1799983 on systolic and diastolic blood pressure.

DISCUSSION

Due to the polygenic character of HTN, it is particularly challenging to identify the single nucleotide polymorphisms most likely to be associated with this condition as well as the connections between certain genes and treatment responses in various ethnic groups [4]. Notably, of the 40% of patients undergoing treatment, almost 65% do not achieve the target <140/90 mmHg [16]. In our cross sectional study, we investigate the association between *eNOS* rs1799983 polymorphisms and EH in Iraqi hypertensive patients. Regarding rs1799983 the most frequent allele was G (73%) while the most frequent genotype was GG (55%) ($p = 0.7$). Our study was demonstrated lack of association between rs1799983 and essential hypertension ($p > 0.05$). Many studies have looked into the relationship between the EH and rs1799983 variant. However, the findings have been inconclusive and controversial. Some studies have found a greater T allele frequency in those with hypertension, and T allele has been linked to resistance to conventional treatment [15, 17]. In contrast, studies conducted on Caucasian groups found a higher frequency of the G allele in the hypertensive group, as well as a relationship between G allele and the outcome, all-cause death [18, 19]. These differences could be a sign that another single nucleotide polymorphism or mutation is connected to one of the two alleles, or they could be a sign that the connections that have been discovered are the result of random errors. On the other hand, other studies reveal a lack of evidence of an association between this polymorphism and essential hypertension in Australians [20] and Japanese [21]. Gamil et al. showed absence of linkage between rs1799983 and essential hypertension among Sudanese people [22].

CONCLUSIONS

Our study is the first in Iraq to investigate the linkage between *eNOS* rs1799983 polymorphism and essential hypertension in Iraqi hypertensive patients. This study concluded the most common allele for rs1799983 was G allele (73%) while the most frequent genotype was GG, frequency of other genotype GT and TT were 36% and 9% respectively. In this study we demonstrate the lack of significant association between two this polymorphism and essential hypertension.

RECOMMENDATIONS

To corroborate this association, additional research with bigger sample numbers and family-based analyses are needed. Future research should concentrate on the interactions between gene-environment and gene-gene, as well as haplotype patterns.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Ghufran K. Salman
University of Al-Qadisiyah
Al Diwaniyah, Al-Qādisiyyah Governorate, Iraq
e-mail: ghufran.k1994@gmail.com

ORCID AND CONTRIBUTIONSHIP
Ghufran K. Salman: 0009-0009-5682-7895 A B
Bassim I. Mohammad: 0000-0001-6732-5940 B F
Hussein A. Saheb: 0000-0002-0137-8932 A D
Ahmed M. Sultan: 0000-0001-6819-0208 C D E
Sinaa Abdul Amir Kadhim: 0000-0001-9375-5581 D E
Asma A. Swadi: 0000-0002-7679-1596 E F

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

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REVIEW ARTICLE

CONTENTS

The private-legal nature of the application of the methods of assisted reproductive technologies in Ukraine

Viktoriia V. Nadon, Arsen N. Isaiev, Olena O. Ruban, Tetiana I. Brovchenko
YAROSLAV MUDRYI NATIONAL LAW UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Aim: Analyze the legislation, judicial practice of Ukraine and EU countries, scientific views on surrogacy, as well as the procedure for legal regulation and registration of the procedure of surrogacy.

Materials and Methods: The following materials were used to write the scientific work: the practice of a number of countries was analyzed; scientific works have been studied; some methods of assisted reproductive technologies are described; the practice of the European Court of Human Rights is analyzed. When conducting the research, a methodology was used that embodies an interdisciplinary approach, which allows for a systematic analysis of theoretical and practical aspects of legal relations arising from the provision of medical services.

Conclusions: At the legislative level, the provision of medical services (surrogate motherhood services) is partially regulated, therefore the basis of the legal relationship between the performers (surrogate mother) and the customers (genetic parents) is the contract concluded and signed by the parties on the provision of surrogate motherhood services. A contract in defined legal relations is a source of law. This contract is bilateral, paid and consensual.

KEY WORDS: embryo, gestational carrier, oocytes, surrogate mother, contract, legal relationship

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INTRODUCTION

In connection with the deterioration of reproductive health (women and men) or for other reasons not related to the health of the married couple, with the emergence of new methods of overcoming infertility, experts increasingly talk about the prospect of the formation of the fourth generation of human rights, related to the preservation of the nation's reproductive health. One of the types of assisted reproductive technologies is surrogate motherhood. Today, this type is very popular. Statistics show that, as a rule, foreign citizens (married couples) turn to this method in countries where surrogacy is either completely prohibited, or this method is expensive, or in order to hide the gestation and birth of their child by another woman.

Surrogate motherhood is prohibited in Germany [1] and a number of other countries such as Switzerland [2], Austria [3], France [4-6], Norway [7], and Italy [8]. Thus, the German Embryo Protection Act aims to prevent split motherhood for the benefit of the child. One argument is that it can harm psychological development and identity during puberty. Advertising and mediation of surrogate mothers is strictly prohibited in this country. In Germany, a woman who has given birth to a child is considered a mother by law. Instead, in Ukraine, a woman can transfer maternity rights to a heterosexual spouse, provided that the child is genetically related to at least one of the ordering parents. A child conceived in this way has three mothers: the egg donor is the genetic mother, the surrogate mother who carries the child and gives birth to it, and after birth the child grows and is raised with the adoptive mother. On 31 August 2023, the European Court of Human Rights ruled on the application in the case of C. v. Italy (application no. 47196/21) on the refusal of the Italian authorities to recognize the family ties established by the Ukrainian birth certificate between child C, born abroad through surrogacy, and her biological father and intended mother. Article 8 of the Convention is at stake. The court recognizes that Italian law does not allow a copy of the intended mother's birth certificate to be made. However, he acknowledges that Italian law guarantees the latter the possibility of legal recognition of the child through adoption. In this regard, the Court notes that, in the opinion of the Plenary Assembly of the Italian Court of Cassation, adoption allows the courts hearing the case to assess the requirements of Article 8 of the Convention and the best interests of the child [9].

Ukraine has become the key country of surrogacy in continental Europe. The rapid growth has coincided

with widespread bans on the practice in India, Cambodia, Mexico and Thailand, after there were increasing scandals over parents not taking away babies born sick.

The crisis in Ukraine fueled the surrogacy business: after Russia occupied Crimea in 2014, ongoing hostilities in the territories of Donbass and Luhansk since 2014, and from 2022 - military operations throughout Ukraine. Accordingly, the country's economy and currency are in free fall, unemployment is high, hundreds of thousands of people are leaving for EU countries, so the reproductive industry is superimposed on the poverty and weakness of the state with a strong tradition of corruption.

According to statistics, in Ukraine, before the war, surrogate mothers gave birth to 2,000 to 2,500 babies a year [10].

The founder and owner of one of the organizations that specializes in providing medical services related to reproductive technologies notes that he controls two-thirds of Ukraine's contracts on surrogacy with customers from other countries. 90 percent of cases were registered by the German Embassy in Kyiv [11].

In 2019, Ukrainian surrogate mothers gave at least 137 babies to fathers with German citizenship (this was reported by the German Ministry of Justice). That is, one child every three days. In 2020, as the pandemic worsened, there were 167 babies born to surrogate mothers who could not be picked up by their genetic parents in time.

Thus, Ukraine is a country where commercial surrogacy is allowed, a country where it is done professionally from a medical point of view, is available in monetary terms, and has a simplified approach to the paperwork procedure (even more simplified during the war). It is these factors that have attracted and continue to attract people who want to have children to choose Ukraine, turning to organizations that offer their services to support and resolve issues related to the selection of a surrogate mother, a medical institution, and legal issues. In fact, the surrogacy program is actually nothing more than trade, since the surrogate mother sells for money the child, whose embryo was given to her by doctors, into the future to her parents, who were found by intermediaries [12].

It should be noted that with the beginning of the war in Ukraine, certain organizations (intermediary organizations on assisted reproductive technologies) suspended the provision of services related to surrogacy, and this is not accidental. Outlined organizations, medical institutions, in which reproductive technologies were introduced, were forced to transport patients (surrogate mothers) to more/less safe cities, so that women could bear and give birth to children. At that time, representatives of organizations acting as mediators in matters of surrogate motherhood were concerned about a number of issues, namely: regarding the evacuation of pregnant women abroad at the request of clients (genetic parents); registration of documents for a child during wartime; transfer and removal of children to the places of residence of genetic parents, etc.

It should be noted that organizations found reasonable answers to all these questions at the legislative level and actively implemented them.

In this regard, from June 2022, clinics resumed their work in safer cities in Ukraine, which were visited by foreign couples who cannot have children due to medical conditions [10]. Surrogate motherhood in accordance with the Procedure for the use of assisted reproductive technologies in Ukraine, approved by the order of the Ministry of Health of Ukraine dated 09.09.2013 No. 787, is defined as one of the assisted reproductive technologies, which allows a couple to become the biological parents of their child if one of them has congenital or acquired diseases that cause infertility [13]. In the Decision of the Court of Appeal of the Kharkiv region in case No. 645/9412/14-ts of June 25, 2015, it is noted that surrogate motherhood services are based on a contractual civil law nature, and in their content are a type of reproductive technologies, when some or all stages of conception and early development of embryos are carried out outside the organism of the genetic mother: «according to the general definition, surrogacy is the process of artificial fertilization, bearing and birth of a child by a woman (surrogate mother) with the aim of transferring the child to the named parents in accordance with a contract for remuneration or without it. A surrogate mother is a woman who voluntarily agreed to become pregnant in order to bear and give birth to a genetically alien child, who will be given to other persons - biological parents for upbringing» [14].

The basis of surrogate motherhood is contractual legal relations, which refer to contracts aimed at providing services, namely medical services.

AIM

Analyze the legislation, judicial practice of Ukraine and EU countries, scientific views on surrogacy, as well as the procedure for legal regulation and registration of the procedure of surrogacy.

MATERIALS AND METHODS

When writing the scientific article, the following materials were used: the practice of a number of countries (USA, Canada, Israel, Italy, the Netherlands, Greece, etc.)

was studied regarding the permission/unauthorization of the use of assisted reproductive technologies – surrogacy; the regulatory framework of a number of countries on the use of assisted reproductive technologies has been studied; used scientific articles by scientists who researched issues on the use of assisted reproductive technologies; an experimental method of reproductive medicine - egg donation - is described; the practice of the European Court of Human Rights in relation to the raised problem is analyzed.

In conducting the research, a methodology was used that represents an interdisciplinary approach, which allows for a systematic analysis of theoretical and practical aspects of legal relations arising from the provision of medical services, namely, the conclusion and execution of a contract on surrogacy, as well as the legal consequences of non-fulfillment/improper fulfillment of assigned obligations ties to the parties. The indicated system of methodology included: historical method, which was applied in the analysis of the transformation of legislative approaches to the regulation of legal relations of surrogate motherhood; systemic - to determine and clarify the legal nature of surrogate motherhood; the formal-legal method was applied in the analysis of the texts of current domestic normative acts, the legislation of the member states of the European Union, which prohibit the procedure of surrogate motherhood, as well as court practice; legal hermeneutics was used to clarify the content of normative legal acts that regulate the procedure of using surrogate motherhood; the comparative legal method was applied to clarify the differences in the legal regulation of the procedure of using surrogate motherhood in Ukraine and foreign countries; the forecasting method made it possible to develop proposals for the elimination of legal conflicts that occur when surrogacy contracts are concluded by foreigners in Ukraine; the dialectical method helped in clarifying the possibility of protecting the rights of children born to surrogate mothers.

REVIEW AND DISCUSSION

In accordance with Article 901 of the Civil Code of Ukraine [15] under a contract for the provision of services, one party (the executor) undertakes to provide a service that is consumed in the process of committing a certain action or carrying out a certain activity, at the request of the other party (the customer), and the customer undertakes to pay the executor for the specified service, unless otherwise stipulated by the contract. From the definition follows the characteristics of the contract for the provision of services, namely, it is bilateral, consensual, in the vast majority, it has a payment nature. However, this does not exclude the possibility of concluding a contract for the provision of services related to surrogate motherhood on a free basis (as is the case in a number of countries of the world, for example, in Belgium, the Netherlands). Altruistic surrogacy is legal in Belgium, but commercial surrogacy is illegal. And although altruistic surrogacy is technically legal, there is only one hospital taking in couples and there are extremely strict rules to get in. This makes a lot of couples seek treatment outside Belgium. Altruistic surrogacy is legal in the Netherland, but commercial surrogacy is illegal. Entering or attempting to enter a surrogacy arrangement can be punished with imprisonment. Moreover, although altruistic surrogacy is technically legal, there are very few hospitals taking in couples and there are extremely strict rules to get in. This makes a lot of couples seek their treatment outside the Netherlands [16]. In Canada, commercial surrogacy is prohibited, although altruistic surrogacy is legal in all states except Quebec [17]. Currently, there is a penalty of four to ten years' imprisonment or a fine of between 250,000$ and 500,000 $ if a person is found guilty of violating Article Five of the Assisted Human Reproduction Law, which prohibits payment for the services of surrogate mothers. Egg and sperm donors are also subject to punishment [18].

In the United States, surrogacy has been permitted and enshrined in law since 1991. It should be noted that this procedure is not the same in all American states. And in some states (Virginia and New Hampshire) surrogacy is completely prohibited by law. In the United States there is no unified legal framework on surrogacy issues. The American Constitution gives states the right to make their own health care laws. For this reason, each state issues its own regulations to address this issue [19].

In Israel, surrogacy is the most preferred solution to the problem of infertility. Thus, in 2018, 128 requests for the surrogacy procedure were considered and only 2 of them were rejected. Surrogacy in Israel is legally permitted with certain restrictions. The most important are the following: previously in Israel, only an unmarried woman could act as a surrogate mother, but 4 years ago the Law "On Contracts for Carrying a Child" was changed. Currently, a married woman can also be a surrogate mother, however, with certain restrictions (a potential "married surrogate mother" must have at least one birth process, but no more than 3). In addition, restrictions from the point of view of legislation include: a couple who decides to resort to the surrogacy procedure must be heterosexual, and a relative of the biological parents cannot act as a surrogate mother [20].

In 1996, the Knesset (Israeli Parliament) adopted the Law "On Contracts for Carrying a Child," which regulates

the rights and obligations of the parties to surrogacy. The most interesting legal provisions are the following: the service cannot be used by unmarried couples and in which the man cannot have children; Only those women who have their own children (from 1 to 4 years old) can bear a child; only those couples who are married to representatives of different sexes and have no other ways to continue the family have the right to apply for surrogacy services [21]. In February 2020, Israel's Supreme Court ruled that restrictions on same-sex couples entering into surrogacy agreements were discriminatory, giving the state one year to change the law. In July 2021, the Supreme Court issued a second ruling stating that the law prohibiting same-sex couples and single men from becoming parents through surrogacy would be invalid for six months. In January 2022, Health Minister Nitzan Horowitz announced that surrogacy would be allowed for same-sex couples, transgender people, and single men as of January 11 [22].

A contract under which the party must receive a fee or other consideration for the performance of its duties is recognized as a paid contract. At the same time, the actions performed by one person (handover of the thing into ownership, use, performance of specified work, provision of services) are compensated by the actions of the other party (payment of the stipulated price, remuneration, etc.). Honiger considered the following to be the conditions of a payment transaction: 1) each of the counterparties is obliged to perform a known action; 2) everyone specified in the contract receives certain actions; 3) the obligation to perform the action of each counterparty depends on the performance of the action by the other counterparty. In contrast to this, the concept of gratuity, according to the author, requires the absence of all the above three points [23].

If unpaid relations lead to a decrease in the volume of the property sphere on one side and to a corresponding increase in it on the other side, then in paid relations - if equivalence is observed - such changes do not occur, and if there is a deviation from equivalence - there are changes in the property spheres, but not in in such striking proportions as in unpaid relations. Payment relations assume a certain ratio between exchanged goods: if equivalence is observed, they are equal; when departing from equivalence, they are not equal. In both cases, a direct connection, characterized by us as a connection in the actions of the participants of the payment relationship, takes place. For each participant, this is expressed in the relationship between the means spent and the results obtained [24]. Some authors concluded that women agree to bear someone else's child mainly in order to improve their financial situation situations [25].

Therefore, legal relations related to surrogate motherhood should be classified as civil law, which are regulated by a contract concluded and signed by the parties. In support of this thesis, it should be noted that draft laws on the regulation of surrogate motherhood in Ukraine have been repeatedly submitted to the Parliament for consideration. In particular, draft laws "On Assisted Maternity" No. 8703 dated 17.06.2011, "On Amendments to Certain Legislative Acts of Ukraine Regarding Restrictions in the Use of Assisted Reproductive Technologies" No. 8282 dated 23.03.2011 (the law was adopted by the VRU in October 2012, but due to the veto imposed by the president it never entered into force), "On assisted reproductive technologies" No. 8629 dated 07.19.2018, alternative project No. 8629-1 dated 08.01.2018 and other draft laws aimed at introducing changes and additions to the current normative legal acts of Ukraine. For one reason or another, there is currently no comprehensive law that would ensure proper state regulation of surrogate motherhood in Ukraine.

Thus, the contract in certain legal relations acts as a source of law. The regulation of social relations by contract was called autonomous regulation as early as the 60s of the 20th century. The importance of the contract is especially increasing under modern conditions, when the contract "not only becomes the main regulator of economic relations, but also acquires the importance of a universal regulator." Expanding the scope of application of legal contracts, as well as strengthening the process of contractual law-making with the development of any of our societies and states, is undoubtedly a progressive phenomenon. In theoretical and practical terms, this means the known limitation of the state monopoly in the field of national law-making and the inclusion of a number of other, non-state entities in this process. A constitutive feature of surrogate motherhood is the conclusion of an agreement on childbearing by a surrogate mother before the child is conceived, with the aim of further establishing parental legal relations between this child and the persons who concluded this agreement with the surrogate mother.

So, at first glance, Ukraine gives the right to use ART, even gives consent to conclude agreements with citizens of other countries, but does not provide the appropriate regulatory framework on this issue. Therefore, all issues must be reflected in the agreement (as a source of law), which is concluded between the surrogate mother and the genetic parents. Therefore, the parties to the said contractual obligation need to be very careful when deciding on the terms of the contract [26]. According to the position of the World Health Organization, which was expressed in Geneva

in 2001, a surrogate mother is a gestational carrier - a woman whose pregnancy has occurred as a result of the fertilization of oocytes belonging to a third party with sperm belonging to a third party. The current legislation of Ukraine does not provide the legal status of a «visiting mother» for a surrogate mother. Establishing such a status is considered inappropriate, given that the child must have one mother, which is completely natural [27]. A woman carries a fetus with the condition that the parents of the child born will be one or both of the people whose gametes were used for fertilization. In this definition, the words "mother" or "parents" are not used, which indicates the contractual nature of the relationship between the customers and the performer.

Thus, in Greece, the implementation of the considered method of assisted reproductive technologies is permitted only with court permission and on the basis of a surrogacy agreement (Article 1458 of the Civil Code) [28]. At the same time, two terms are used in Greek legislation: "gestational motherhood" (gestational motherhood) and "surrogate motherhood" (substitute motherhood). The first term refers to a situation where a woman (relative, friend or stranger) proposes to transfer into her uterus an embryo obtained in vitro from a pair of gametes (the applicant or assignee). In characterization, it can also be thought of as a "loan" or "womb rental." The second term refers to situations in which the woman provides both the eggs and the uterus. A woman gives birth to a child on the side on which he is "ordered". In fact, the second situation is prohibited by Greek law, but "womb rental" is permitted: "The transfer of a fertilized egg to another woman and her pregnancy with the permission of the court, if prior to the transfer there is a written agreement between the parties wishing to have the child and the surrogate mother, provided that the latter also is married. The court's permission is issued after filing an application wishing to have a child, provided that due to the circumstances of the circumstances she is not capable of conceiving a child" (Article 1458 of the Greek Civil Code). Yes, the right to appeal in court, only the actual mother is available for data if she has a medical certificate confirming that she is infertile and (or) there is a transmission of a severe hereditary disease to the child (for example, the risk of anemia) (Article 1455 of the Civil Code of Greece). Greek legislation stipulates that a surrogate mother must undergo not only a medical examination, but also a serious psychological examination. In court, a surrogacy contract is drawn up, which is concluded in writing and signed by the actual and surrogate mother, as well as their spouses (if any) [29, p. 282-287]. Thus, by signing this agreement, the parties confirm their consent to ensure surrogacy, as well as respect for parental rights of genetic parents after the birth of the child. In addition, in Greece, as in the UK, there is monetary compensation only for the current expenses ("reasonable expenses") of the surrogate mother.

In accordance with Ukrainian legislation, under the agreement on surrogacy, the surrogate mother (the performer) gives her consent to the fact that the embryo of the married couple (the customer) will be implanted in her body and, accordingly, assumes the responsibility for bearing and giving birth to the child, and the customer undertakes to pay all the stipulated costs . The cost of surrogacy in Ukraine today starts at 50,000 Euros. This amount includes: 1) organizational costs (meeting customers, hotel accommodation, food, etc.); 2) expenses related to the selection of a surrogate mother (medical examination, introduction of material into the woman's body, etc.); 3) expenses related to the normal course of pregnancy (proper nutrition, accommodation, treatment, medical examination, etc.); 4) expenses related to the remuneration of the surrogate mother (executor) for the proper performance of the duties assigned to her, related to the proper bearing and birth of the child; 5) expenses related to the preparation of documents.

Thus, with the consent of a woman to become a surrogate mother, she is solely entrusted with the duties that are prescribed in the contract and which she must fulfill properly. If the surrogate mother performs her duties improperly, then she will not receive the remuneration stipulated in the terms of the contract (remuneration for carrying and giving birth to a child). As for the customers (a married couple), they have a single obligation, namely: covering all agreed costs.

Surrogate mothers can be women who have given voluntary consent. Article 48 of the Fundamentals of the Legislation of Ukraine on Health Care [30] provides that the use of artificial insemination and embryo implantation is carried out according to the medical indicators of an adult woman with whom such an operation is performed, subject to the written consent of the spouses, ensuring the anonymity of the donor and maintaining medical confidentiality. These women can be relatives or acquaintances of an infertile couple, those who wish are specially examined. The requirements for surrogate mothers include: age (from 18 to 36 years old; (in Greece up to 50 years of age)), having a healthy child of their own, mental and physical health. A surrogate mother cannot be an egg donor at the same time. A married woman can be a surrogate mother only with the written consent of her husband. Part 2 of Art. 139 of the Civil Code of Ukraine establishes a ban on contesting maternity in cases where the procedure of transferring a

human embryo conceived by a couple as a result of ART was carried out into the body of another woman [31].

Most of the parents-customers, as practice shows, are over 50, some even over 60 years old. Customers are offered a menopause withdrawal service. This is an experimental method of reproductive medicine: mitochondrial donation. Thus, egg donation can be genetically disguised and physical capacity can now be created not only for the father, but also for the mother. Mitochondria – cell bodies with their own genetic material – are removed from the eggs provided by the woman, and mitochondria from the customer's mother are used instead. If a child was born from a fertilized egg that contained genetic material from another woman, a DNA test will not show that the two mothers were related before conception. This method is being experimented in one of the clinics in Ukraine. However, the use of mitochondrial donation is subject to criticism.

A counter argument might be posed that the decision to use a certain reproductive option is a down to personal choice, and for some patients, being genetically related to the child will influence this decision. To support this, recent HFEA figures show that ~5% of IVF cycles performed in the UK in 2014 used donor eggs [32]. This implies that most couples opting for fertility treatment choose to pursue a reproductive option that allows them to have a genetically related child when such an option exists

Therefore, surrogate motherhood is a type of Assisted Reproductive Technology (ART), in the case of which a woman (surrogate mother) voluntarily agrees to become pregnant in order to carry and give birth to a child biologically alien to her, who will then be transferred to other persons (genetic parents) for upbringing, and with whom the surrogate mother will no longer be bound by either rights or obligations.

In the decision of the ECtHR in the case "Labassee v. France"[33] it is noted that each state can independently decide on the issue of allowing or prohibiting surrogate motherhood on its territory. In this regard, scientists note that although the right to reproductive choice is provided for by international and regional normative legal acts, in some cases, national legislation prescribes restrictions on the exercise of reproductive rights [34].

In the case of the birth of a child by a woman into whose body a human embryo, conceived by a spouse as a result of ART, was transferred, the state registration of the child's birth is carried out at the request of the spouse who gave consent to such a transfer. In such a case, together with the document confirming the fact of the birth of a child by this woman, a statement is submitted about her consent to the registration of the marriage by the child's parents, the authenticity of the signature, which must be notarized, as well as a certificate about the genetic relationship of the parents (mother or father) with the fetus. Thus, the genetic parents acquire legal rights over the newborn child [35, 36].

It should be noted that on March 4, 2022, the Ministry of Health of Ukraine approved the order "Regarding registration of newborns under martial law" [37]. The adopted decision allows to register the birth of a child if the birth took place outside the health care facility. In this case, a document on the birth of a child in the form of a medical birth certificate (form No. 103/o) can be issued by medical workers who were present during the birth or conducted the first examination of the newborn.

If the genetic parents do not dare to come to Ukraine in person, heeding their own government's advice about the risks of travel during martial law, there is the possibility of obtaining a birth certificate from their duly authorized representative in Ukraine. For this, after the birth of a child, the spouses of the genetic parents must take certain actions at their place of residence or stay, namely: issue notarized copies of passports, power of attorney for a representative and an application for registration of the child's birth; submit these documents to the competent authorities of the state where they were signed for affixing an apostille or passing the consular legalization procedure; send original documents to Ukraine.

The issue of legalization of the legal status of a child born to a surrogate mother has already been raised at the level of decisions of the European Court of Human Rights. According to the precedent law of the European Court, Art. 8 of the Convention requires that national legislation provide for the possibility of recognizing the legal relationship between a child born under surrogacy arrangements concluded abroad and the intended father, if he is the biological father. The lack of such an opportunity entails a violation of the child's right to respect for private life, guaranteed by Art. 8 of the Convention.

The European Court considers that the general and absolute lack of possibility to obtain recognition of the relationship between a child born under surrogacy arrangements concluded abroad and the intended mother is incompatible with the best interests of the child, which require at least that each situation be considered with taking into account the special circumstances of the case.

On April 10, 2019, the European Court of Justice prepared an advisory opinion at the request of the French Court of Cassation regarding the recognition in national law of legal relations between parents and a child born abroad in accordance with an agreement on gestational surrogacy.

In its opinion, the European Court observed that the right to respect for the private life of a child born abroad

under a gestational surrogacy agreement requires that national law provide for the possibility of recognizing the legal parent-child relationship between the putative mother designated as the "legal mother » in a birth certificate officially issued abroad.

From the point of view of international private law, in the case of the birth of a child by a surrogate mother - a citizen of Ukraine abroad, the law of the country of the place of transfer of the embryo(s), i.e. Ukraine, cannot be used to regulate the issue of establishing paternity. In such cases, determining the origin of a child born as a result of using the surrogacy method from parents of other countries in the territory of a third country requires clarification of conflicting connections. And of course, the main reference for solving this issue will be the personal law of an individual (lex personalis), which determines the legal status of an individual, that is, the law of the state of which he is a citizen. Given the specificity of the legal relationship of surrogacy, it will be the personal law of one or both genetic parents, unless the child was born in a country that recognizes the acquisition of citizenship by birth (for example, the USA). The legislation of certain American States are sufficiently progressive in the context of legal regulation of surrogacy and its consequences. Taking this fact into consideration it can be used as a sample for legislation of other countries [38].

CONCLUSIONS

1. At the legislative level, the provision of medical services (surrogate motherhood services) in Ukraine is partially regulated, therefore the basis of the legal relationship that arises between performers (surrogate mother) and customers (genetic parents) is the contract concluded and signed by the parties on the provision of surrogate motherhood services. According to the contract on surrogacy, the surrogate mother (the performer) gives her consent to the fact that the embryo of the married couple (the customer) will be implanted in her body and, accordingly, assumes the responsibility for bearing and giving birth to the child, and the customer undertakes to pay all the stipulated costs. Costs related to the proposed service should be divided into: 1) organizational costs (meeting customers, hotel accommodation, meals, etc.); 2) expenses related to the selection of a surrogate mother (medical examination, introduction of material into the woman's body, etc.); 3) expenses related to the normal course of pregnancy (proper nutrition, accommodation, treatment, medical examination, etc.); 4) expenses related to the remuneration of the surrogate mother (executor) for the proper performance of the duties assigned to her, related to the proper bearing and birth of the child; 5) expenses related to the preparation of documents.
2. A contract in defined legal relations is a source of law. This contract should be characterized as bilateral, paid and consensual. Therefore, surrogate motherhood in Ukraine should be clearly regulated at the legislative level, first of all, the interests of the child, and not someone else, should be taken into account. In this context, the legalization of the legal status of a child born to a surrogate mother needs clear regulation.
3. Having analyzed the legislation of a number of countries in which surrogacy is prohibited or limited, the author of the article comes to the conclusion that in Ukraine it is worth limiting surrogacy and moving away from commercialization (surrogacy tourism). Children are not a commodity; they cannot be sold. In exceptional cases, assistance to a healthy woman in carrying and giving birth to a child may be allowed to a family that, for medical reasons, cannot have children, but exclusively on a free of charge basis, as provided in a number of countries.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Viktoriia V. Nadon
Yaroslav Mudryi National Law University
77 Pushkinska St., 61024 Kharkiv, Ukraine
e-mail: nadonviktoria@gmail.com

ORCID AND CONTRIBUTIONSHIP

Viktoriia V. Nadon: 0000-0001-8240-7717 A D E
Arsen N. Isaiev: 0000-0002-9982-0572 A B
Olena O. Ruban: 0000-0002-8602-0517 D E
Tetiana I. Brovchenko: 0000-0002-2095-8887 C E F

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

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REVIEW ARTICLE

CONTENTS

Portal hypertension and emergency care

Diana V. Rudyk, Mykola I. Tutchenko, Sergiy L. Chub, Mykola S. Besedinsky
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE,

ABSTRACT

Aim: To evaluate the peculiarities of the course of complications and the provision of care for portal hypertension associated with the development of diuretic-resistant ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, and variceal bleeding.

Materials and Methods: This research is based on a review of the literature in PubMed, CrossRef, Google Scholar sources on complicated portal hypertension. Such complications of portal hypertension as spontaneous bacterial peritonitis, ascites, hepatorenal syndrome, variceal bleeding caused by sinistral portal hypertension are considered. The effectiveness of interventional treatment methods and laparoscopic surgical interventions has been demonstrated.

Conclusions: Diagnosis and treatment of patients with complicated portal hypertension requires a multidisciplinary approach, which is due to the diverse pathophysiological process of portal hypertension. The possibilities of providing emergency care to this category of patients depend on the level of medical training of the staff, the possibilities of medical and technical support in the provision of interventional care, the ineffectiveness of which necessitates surgical treatment using minimally invasive technologies.

KEY WORDS: portal hypertension, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, bleeding

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INTRODUCTION

Portal hypertension, which occurs when the pressure gradient in the portal system exceeds 6 mm Hg, gradually leads to the development of complications that are accompanied by hospitalization and threaten the life of the patient. Complications in the form of ascites, hepatorenal syndrome, bleeding from the veins of the esophagus, stomach and other parts of the digestive tube, spontaneous bacterial peritonitis are difficult to diagnose and often require emergency care. Medical assistance for complicated portal hypertension is versatile, including drug support, interventional treatment, in case of ineffectiveness of which, surgical intervention is performed. The only radical method of treatment for patients with decompensated portal hypertension is liver transplantation.

AIM

To evaluate the peculiarities of the course of complications and the provision of care for portal hypertension associated with the development of diuretic-resistant ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, and variceal bleeding.

MATERIALS AND METHODS

This research is based on a review of the literature in PubMed, CrossRef, Google Scholar sources on complicated portal hypertension. Such complications of portal hypertension as spontaneous bacterial peritonitis, ascites, hepatorenal syndrome, variceal bleeding caused by sinistral portal hypertension are considered. The effectiveness of interventional treatment methods and laparoscopic surgical interventions has been demonstrated.

REVIEW AND DISCUSSION

Portal hypertension (PH), which is unceasingly developing, is caused by the formation of complications, among which ascites is the primary one that needs care.

PH and ascites are two crucial events in the natural history of liver cirrhosis, whose appearance marks a downward shift in the prognosis of the disease and over the years, several international and national societies have issued clinical practice guidelines for the diagnosis and management of PH and ascites [1].

The Japanese guidelines are based on the updated clinical guidelines for the treatment of cirrhosis and ascites, which are compared with the guidelines of Eu-

rope and the United States for the treatment of grade one to three ascites [2- 5].

Ascites — an abnormal accumulation of fluid in the peritoneal cavity — is present in up to 90% of patients with advanced cirrhosis, resulting in frequent hospitalisations due to debilitating episodes of pain and breathlessness [6]. About 20% of patients with cirrhosis have ascites at their first presentation, and 20% of those presenting with ascites die in the first year of the diagnosis [7].

Among the various risky complications of liver cirrhosis, refractory ascites is associated with poor survival of cirrhotics and persistently worsens their quality of life [8].

Palliative long-term drainage of the abdominal cavity in patients with cirrhosis with refractory ascites has an advantage over therapeutic paracentesis and consists on placing a tunnelled drain through the abdominal wall, with ultrasound guidance, and with the patient under local anaesthetic [9,10].

One of the essential points caused by ascites is an increase in pressure in the abdominal cavity, which leads to the deterioration of abdominal perfusion.

In critically ill patients, abdominal perfusion pressure (APP), resulting from the difference between mean arterial pressure and intra-abdominal pressure (IAP), correlates with improved survival however, there are reports of different clinical threshold values for APP ranging from 50 to 72 mm Hg [11]. Chronically increased IAP is present in the physiological state of pregnancy and in pathological states such as morbid obesity, decompensated heart failure and liver cirrhosis [12].

Less with that APP is a critical vital sign that assists the clinician in titrating volume restoration, administering vasopressors, and optimizing intra-abdominal pressure, thereby preventing the deleterious effects of persistent critical pressure [13].

The researchers concluded that APP can be considered a critical sign, so prevention and treatment of abdominal hypoperfusion in patients with decompensated cirrhosis is urgent, and mortality is higher among patients with hypoperfusion, independent of factors such as clinical severity, total bilirubin, and hepatic encephalopathy [14,15].

An increase in portal pressure, due to both the sinusoidal and presinusoidal components with the accumulation of ascitic fluid, leads to swelling of the intestinal wall and is accompanied by a violation of its permeability with subsequent formation of spontaneous bacterial peritonitis (SBP).

SBP is a term used to describe acute infection of ascites, an abnormal accumulation of fluid in the abdomen without a distinct or identifiable source of infection [16]. Ascites is a predominantly transudative fluid with weak opsonic activity, providing a favorable environment for bacterial growth in 10–30% of hospitalized patients [17].

A diagnosis of classic SBP is made if neutrophils count in the ascitic fluid is ≥250 cells/mm3, culture results are positive, and secondary causes of peritonitis are excluded [18]. SBP is most commonly (75%) caused by gram-negative aerobic organisms, with *Klebsiella pneumoniae* accounting for 50% of these, gram-positive aerobic microorganisms are responsible for the remainder of cases; the most common of these are *Streptococcus pneumoniae* or *Viridans group streptococci* [19].

Patients with SBP have Child-Pugh Class C chronic liver disease, have a 1-year survival rate of 45% and a 2-year survival rate of 35% [20].

In hospital settings, the non-infection-related mortality in SBP patients can be as high as 20-40 percent, and one to two years of mortality rates are 70 and 80 percent, respectively [21].

Thus, ascites, which complicates the course of portal hypertension, leading to an increase in intra-abdominal pressure, becomes even more dangerous for the patient's life due to its infection and requires treatment of both ascites itself and its bacterial component, as well as correction of intra-abdominal pressure. Another complication that occurs in patients with decompensated PH and ascites is hepatorenal syndrome (HRS), the provision of care for which is an extremely difficult task.

Renal failure is the leading cause of death in patients with SBP and develops in 30–40 percent of patients, but risk can be minimized by treatment with octreotide or midodrine is helpful if renal failure develops [22].

Until recently, HRS was categorized into 2 major types: the severe and rapidly progressing form of HRS (formerly called HRS type 1 [HRS-1]) marked by cirrhosis with ascites, and the milder, more slowly progressing form of HRS (formerly called HRS type 2 [HRS-2]) that is typically associated with refractory ascites [23,24].

HRS-acute kidney injury (AKI) is associated with a high mortality rate and can occur due to a precipitating event like bacterial infection and/or following acute liver injury stemming from causes such as alcohol abuse, drug use, hypovolemia from overuse of diuretics, and exacerbations of viral hepatitis [25,26].

AKI due to HRS has the highest short-term (30 days) mortality followed by AKI due to infection in decompensated cirrhosis and detection of AKI using creatinine kinetics-based definition may prompt an early appropriate intervention [27].

Preventive measures against the development of AKI in cirrhosis include avoidance of potentially nephrotoxic medications like nonsteroidal anti-inflammatory drugs, avoidance of excessive or unmonitored diuretics or nonselective beta-blockade, avoidance of large-volume para-

centesis without albumin replacement, and counseling patients to avoid alcohol use [28]. HRS patients treated with terlipressin experienced better clinical outcomes and a lower cost per treatment response vs other unapproved treatments [29-31].

Ascites, SBP, HRS, these complications of decompensated PH require constant monitoring with the need to provide emergency care, which is often provided with medication, while bleeding from varicose veins requires interventional or surgical measures.

Separately, sinistral portal hypertension (SPH) is distinguished, which is defined as increased pressure limited to the gastrosplenic side of the portal vein system and differs from other forms of portal hypertension in that liver function is preserved, the portal vein is open and treatment requires a multidisciplinary approach with surgery and interventional radiology [32]. For interventional radiology belongs splenic artery embolisation which is an effective treatment for gastric variceal bleeding secondary to SPH [33].

Esophageal variceal bleeding (EVB) is one of the most severe adverse events of liver cirrhosis [34].

Seven percent of patients with liver cirrhosis develop the symptom of esophagogastric fundic varices each year [35].

Patients with cirrhosis who are not treated prophylactically have a high risk of rebleeding within 1 year and a mortality rate of approximately 15%–20% within 6 weeks [36].

In recent years, some evidence suggests that endoscopic variceal ligation combined with endoscopic sclerotherapy can significantly reduce bleeding and recurrence of esophageal varices, with similar adverse events compared to endoscopic varices ligation alone [37,38].

Gastrointestinal bleeding is often encountered in patients with cirrhosis, with an incidence of 85% in Child Class C versus 45% in Child Class A and patients in Child Class C also have up to 30% mortality risk compared to other classes [39].

Gastric varices infrequently bleed compared to oesophagal varices, they may lead to massive bleeding resulting in hemorrhagic shock and death in 25-55% of cases due to the rapid flow of blood in the varices and the inflow and outflow vessels [40].

Balloon-occluded retrograde transvenous obliteration(BRTO) appears feasible and successful in patients with poor hepatic functional reserve or hemorrhagic diathesis.

Transjugular intrahepatic portosystemic shunt (TIPS) is a nonsurgical intervention to reduce portal pressure by creating a low-resistance channel between the portal and systemic circulations.

TIPS can significantly decrease the level of the portal pressure gradient (PPG), but it is only considered a treatment choice after failed first-line treatment.

Patients with a portal venous pressure ≥25 mmHg or at high-risk of the first-line treatment failure and mortality, the primary goal is to reduce the level of portal venous pressure, which indicate TIPS can be applied as a first-line treatment to prevent further life-threatening conditions.

TIPS treatment has been extensively used in the treatment of variceal bleeding for more than two decades; however, TIPS treatment increases the incidence of hepatic encephalopathy, which was reported as 16% to 31% in post-TIPS patients.

Gauri Mukhiya et al, evaluated the efficacy and safety of TIPS combined with gastric coronary vein embolization (GCVE) for cirrhotic portal hypertensive variceal bleeding and compare outcomes of first-line with second-line treatment, coil with glue, and single-covered with double stents.

However, whether TIPS or BRTO is more beneficial for GVB patients, especially regarding the overall survival rate, still needs to be discovere.

Mini-invasive surgical technologies expand the possibilities and are successfully used in the treatment of patients with portal hypertension and its complications.

During the last three decades the Sugiura procedure and other nonshunting operations have been widely performed as the operations of choice for bleeding esophageal varices in Japan.

Surgery is usually performed in patients with PH with a high risk of variceal bleeding in China using individualized and precise total laparoscopic surgical procedure based on 3D remodeling for PH.

Esophagogastric devascularization and splenectomy are safe and effective to treat PH secondary to liver cirrhosis and it can be performed successfully in elderly patients and achieve a curative effect that is not inferior to young patients. Laparoscopic Hassab's procedure is a feasible treatment for esophagogastric varices with PH in terms of both short- and long-term results.

Thus, PH occurs when hemodynamics is disturbed in the portal system and is accompanied by the development of complications that potentiate each other and negatively affect the results of diagnosis and treatment.

CONCLUSIONS

Diagnosis and treatment of patients with complicated PH requires a multidisciplinary approach, which is due to the diverse pathophysiological process of portal hypertension. The possibilities of providing emergency care to this category of patients depend on the level of medical training of the staff, the possibilities of medical and technical support in the provision of interventional care, the ineffectiveness of which necessitates surgical treatment using minimally invasive technologies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Diana V. Rudyk
Bogomolets National Medical University
13 T. Shevchenko Boulevard, 01601 Kyiv, Ukraine
e-mail: Dianavrudyk@ukr.net

ORCID AND CONTRIBUTIONSHIP

Diana V. Rudyk: 0000-0001-9736-3614 A B E F
Mykola I. Tutchenko: 0000-0002-5050-6494 A D E F
Sergiy L. Chub: 0000-0001-6489-7345 B F
Mykola S. Besedinsky: 0009-0009-8618-1458 A B

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REVIEW ARTICLE

CONTENTS

Superconscious processes as mental images of a higher order (in the context of telezombification)

Vitalii Shmarhun[1], Denys Shpak[2], Lidiia Spivak[3,4], Svitlana Shkil[5], Ihor Hoian[6]

[1]NATIONAL UNIVERSITY OF LIFE AND ENVIRONMENTAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[2]MYKOLAIV REGIONAL INSTITUTE OF POSTGRADUATE PEDAGOGICAL EDUCATION, MYKOLAIV, UKRAINE
[3]IVAN ZYAZYUN INSTITUTE OF PEDAGOGICAL AND ADULT EDUCATION, NATIONAL ACADEMY OF EDUCATIONAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[4]UKRAINIAN INSTITUTE OF ARTS AND SCIENCES, BUCHA, UKRAINE
[5]DRAHOMANOV NATIONAL PEDAGOGICAL UNIVERSITY, KYIV, UKRAINE
[6]VASYL STEFANYK PRECARPATHIAN NATIONAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: The paper aims to examine superconscious processes as mental images of a higher order in the context of telezombification.

Materials and Methods: The authors used interpretive research paradigm, psychoanalysis, basic principles of hermeneutics, phenomenological approach along with general scientific methods, such as induction, deduction, generalization, etc.

Conclusions: With the beginning of the russian full-scale attack on Ukraine, russian atrocities in Bucha, Mariupol and other cities and villages of the country, many Ukrainian citizens asked about what has happened to the russian society, the state authorities, who set the goal of destroying Ukraine as a state and all its inhabitants as a nation. Then Ukrainians have labelled the invaders and the authorities of Russia as non-humans. And this is a fair name for them. The fact is that these occupiers and their neo-Nazi leaders have a destroyed, distorted consciousness as a result of which they became incapable of realizing their thought processes. The consciousness of such persons gradually degrades towards animal thinking, the so-called proto-thinking. This is one direction to not realizing one's intentions and actions at the level of both subconscious and partially conscious analysis of primary mental images (images of a first and second orders). The second direction is the role of superconscious processes, in particular mental images of a higher level, which also form the worldview positions of an individual in the process of viewing and listening to certain information, while remaining, at the same time, unconscious until a certain time. Together, these directions form a person's attitude to existing social and worldview problems.

KEY WORDS: consciousness; psyche; superconscious; psychic images; high-order images; russian-Ukrainian war; telezombification; telezombified personality

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INTRODUCTION

Given the title of the paper, there is a need to explain the modern approach to the concepts of subconscious and superconscious. To date, the problem of the superconscious has been studied much less than the problem of the unconscious and subconscious. The term "superconscious" was introduced in psychology in contrast to the popularity of theories of the subconscious, but a strict division of the concepts has not been carried out yet. If in relation to the concepts of subconsciousness and superconsciousness, they are spatially separated (one is "under" and the other is "above"), then in relation to the concepts of "unconscious", "subconscious" it is more difficult to do this. First of all, let's briefly define these concepts. The unconscious in this sense is perceived as a set of mental states and processes that are carried out without consciousness. That is, the entire set of unconscious processes is called unconscious in general.

"Subconscious" we understand as the mental material that is not currently conscious, but which in principle can be conscious or was once conscious. This also includes the classic unconscious according to Z. Freud [1] – mental material that was repressed due to its incompatibility with human moral values. The concept of "unconscious" is broader: it includes both the unconscious and psychic material that has never been and may never be conscious (for example, K. Jung's collective unconscious) [2].

In the mental sphere, Z. Freud distinguishes three areas according to the hierarchical principle: subconsciousness, consciousness and superconsciousness. The subconscious of the unconscious appears, on the one hand, as a naturally determined component of the psyche, and on the other – as a component, the content of which is determined by the individual's past experience. The superconscious of the unconscious are factors determined by the structure of the cultural space, in which an individual operates, which are realized but not regulated psychologically. For example, a person speaks without paying attention to the principles of the functioning of language; participates in political actions discovering later that their results were something far beyond his/her intentions and goals; uses devices often without knowing anything about their development.

The subconscious is a psychological system that is located in the deep layers of the human psyche and is manifested only in extraordinary cases and with the help of special techniques. The deep layers of the psyche are what is "under" consciousness, that is, located in the depths of the psyche, directly "under consciousness". The subconscious mind differs from consciousness in that the reality reflected by it merges with the subject's experiences, his/her attitudes, therefore, arbitrary control over the actions performed by an individual and evaluation of the results are impossible in the subconscious. In the subconscious, the past, the present, and the future often coexist uniting in a single mental act (for example, in a dream). The term "subconscious" was used by P. Janet [3, 4] for designation of mental processes that flow without reflecting them in consciousness and outside conscious control.

The subconscious mind is mental material that is outside of consciousness, but is involved in the regulation of behavior, so it is accessible to objective study and awareness. The unconscious is the content of the psyche where virtual images, emotions and urges arise, which can potentially become regulators of behavior or the content of dreams, but can disappear forever. Everyday experience passes through awareness, and everything that is perceived is stored in consciousness, that is, consciousness is conscious experience. Everything that we are not aware of enters the subconscious.

Between the conscious and the subconscious there is a "Keeper" that maintains the balance between these two structures. In addition, the experience is also fixed in the body, since the latter has a kind of memory. The higher "I" is an intuitive personal beginning that performs integrative and coordinating functions between the specified structures (conscious, subconscious, body) and connects this system with the surrounding world. R. Assagioli, the founder of the psychosynthesis, set the goal of bringing the versatile material of the unconscious to consciousness integrating it and achieving a synthesis of this material with consciousness in the process of self-discovery [5].

The division into subconsciousness and superconsciousness is carried out according to a different principle. Both can refer to the unconscious as a whole, and to its unconscious part, in other words, it can be potentially accessible to consciousness or it can be in such depths that the light of consciousness may not reach. The main difference between the subconscious and superconscious, in our opinion, lies in the plane of time. The subconscious as actual mental information is in the past, it does not matter whether we understand it in Freud's terms as repressed from consciousness (that is, the former was previously conscious) or as Jung's collective unconscious (which will never appear in individual consciousness) – it's all in the past. The collective unconscious is a kind of ancestral memory. It is mainly represented by early childhood experiences. The superconscious is in the future – it is something that will be realized in the future as meanings, ideas, and experiences.

The abovementioned is important for theoretical scientific psychological discussions about the meaning of concepts, definitions, etc., as well as for practical implications, first of all, for the awareness of many social processes taking place around us: how to form our attitudes towards them; our social positions; our place and role as the co-participants and creators of these processes; our awareness of ourselves as personalities; our responsibility for everything that happens to us. Everything that was mentioned closely related to the superconscious processes of the formation of the high-order images (HO), which a person perceives from the TV screen in visual and verbal form. Such images tend to affect the worldview of an individual and determine his/her attitude to everything that happens in society [6, 7].

AIM

The paper aims to examine superconscious processes as mental images of a higher order in the context of telezombification.

MATERIALS AND METHODS

The authors used interpretive research paradigm, psychoanalysis, basic principles of hermeneutics, phenomenological approach along with general scientific methods, such as induction, deduction, generalization, etc.

REVIEW AND DISCUSSION

Psychologists wrote about the connection between the superconscious and the future at the end of the last century. It mostly concerned scientific and artistic creativity. For example, K. Stanislavsky singled out a special component of the creative process and called it "superconsciousness" – the highest level of the creative process, which differs from both its conscious and unconscious components. Scientific or artistic creativity is always directed to the future, the act of enlightenment itself does not complete, but only initiates the activity of creating a work, and the work gives rise to numerous social and cultural processes that will continue. Over time, along with artistic and scientific creativity, love, the choice of a profession, the choice of one's point of view about the world: one's place in society, the meaning of one's life (how I live, whether I live with dignity, the faithfulness of my decisions, what awaits me and my children in the future, my responsibility for supporting and promoting anti-human, anti-social ideas, etc.). It is easy to see that these are also phenomena that begin in the present and stretch into the future [8]. Love leads to the creation of a family, the birth of children, i.e., new generation. The chosen profession allows people to realize their potential in the future. The social position and responsibility (as a social creator) will also necessarily manifest itself in the future.

The next difference between the superconscious and the subconscious is that superconscious processes always lead to the creation of a new mental product, which did not exist before. The difference between these two levels (subconscious and superconscious) can be explained by a simple example. In the process of recalling any event, an individual reproduces previously learned information that is stored at the subconscious level. Solving a creative or social (political) task, a person creates new information that did not previously exist either in his/her individual or social experience. It is realized after it was acquired (though not always in its true sense). In this case, the work of thought is carried out at a superconscious level.

And the other difference is related to the cognitive processes that underlie this phenomenon. The superconscious turns out to be connected to thinking processes, because thinking is engaged in solving problems, testing hypotheses, and generating a new product. So, the activity of superconsciousness is the activity of thinking, which is not realized, but remains a cognitive process. If we talk about the subconscious in terms of cognitive processes, it is rather memory.

As a possible mechanism of the work of the superconscious, the formation of mental images of the real HO can occur. Until now, researchers described two groups of mental images: primary and secondary (perceptions and representations) [9]. The first ones are perceptions. The second are the images that are in our memory, the represent specific objects and phenomena. For instance, these are the images of a house, a tree, a parent or a relative, the stages of our childhood, etc. These are the images of the second order. The HO images are formed based on the primary and secondary ones in the psyche [10]. They are formed as a result of the accumulation of scientific information, which is located in the images of the lower order. Our model is close to the concept of memory as dual encoding – this information will be realized in the future in the form of meanings, ideas, and experiences.

In many theories of consciousness, the "sensory fabric of the image" is included in the structure of consciousness, and these are the images of perception (primary images, and in our terms – images of zero order). Presumably, the images included in consciousness without an external stimulus will be secondary, that is, concrete pictures or sounds. Therefore, in order to become conscious, in most cases the HO image must perform an operation that is the opposite of generalization, i.e. anti-prototyping: from the multitude of generalized images, one of a lower order will be selected, and it will be the one that will become part of the act of consciousness and be associated with the word [10].

In any case, we can influence the content of moral, aesthetic, and intellectual standards by repeatedly showing a person the image of the desired object. The possibility of such a phenomenon, at least for some low-order images, is proved by the effectiveness of modern means of propaganda. In fact, the TV screen bombards a person with the images of success, wonderful things or enemies and horrors. It is believed that the effectiveness of propaganda through the imposition of the HO images is not even in the fact that a person will remember information, but in the fact that it will be generalized and integrated already without the participation of human consciousness forming new standards and patterns of behavior. At the same time, it does not matter whether a person agrees with the displayed information or is outraged by it.

Therefore, the awareness of the HO images, in contrast to verbal logical thinking, the generalization of visual information in a person, occurs without the participation of consciousness. And this is important to remember. In general, the creative productivity of a person depends on two factors. The first is the formation of an "appropriate" HO image. The second is awareness of the information contained in the HO image. However, even goals, motives and psychological qualities are not always consciously formed by a person, but the

influence of personality on conscious processes still exists. Let's repeat that during the formation of the HO images, there is a generalization of visual information received from the outside world.

Let's note once more that the HO images are formed beyond the control of consciousness. They are formed in the process of summarizing visual information coming from the senses. Images of the second order are classic secondary images – images of specific objects; images of the third order are images of the generalization of objects, their visualization as a contour; images of the fourth and fifth order are representations of the highest level of generalization, which are spatial and are rooted in the visual world. The subconscious is associated with the images of the second or third order, which represent in a figurative form the memory material repressed from consciousness.

Superconsciousness is associated with the formation of the images of the fourth and fifth orders. Here we can highlight four functions of such images in superconscious processes. Firstly, such images can be unconscious moral, philosophical, and psychological standards or mathematical generalizations. Secondly, they can be formed in the process of solving certain tasks (for example, to watch or not to watch war propaganda) as unconscious gestalts that summarize all available information. Thirdly, in the HO images, a person can elaborate ideas for artistic or scientific works, "guidelines" for understanding the world around us. Fourthly, high-order images can "shape" the processes of intuition as long as they remain unconscious.

It should be noted that the relationship between the images of the superconscious and consciousness is quite complex. Despite the fact that the HO images are unconscious, their formation is influenced by the quantity and quality of perceived visual information, as well as the motives, goals, and desires of a person. The easiest way to manage the unconscious formation of such an image is through the correct selection of incoming information. Firstly, human life experience is different, people live in different cities, meet different people, choose different professions – all this determines their image of the world, which is individual for each person, despite the fact that the world around us is one-unified. Secondly, the well-known phenomenon of the "selectivity of perception": a person in the surrounding world mostly "sees" what corresponds to his/her interests and values and does not notice what does not fit into his/her picture of the world. It turns out that, on the one hand, the selectivity of attention is determined by personal characteristics and, on the other hand, it shapes these characteristics of the personality [10].

Generalization and integration will take place without human will, and therefore, after some time, his/her value orientations will still begin to change. The proof is the effectiveness of intrusive advertising: regardless of our dissatisfaction by the very fact of advertising and the displayed goods, the level of sales increases after each advertising campaign. However, even with the help of such massive imposition of images, non-verbal patterns can only be slightly transformed, but not fundamentally changed, since visual information comes not only through the mass media. For the HO images, which are in the process of solving certain tasks, the personal factor is more significant than the images of standards. But the role of the individual's consciousness as a whole is still limited to setting goals, motives and selecting information.

The first thing a person can do is to set a goal, for example, not to watch and listen to the russian TV presenter O. Skabeyeva. After all, only an individual determines whether he/she needs to think about what he/she has heard and seen and change something in his/her worldview. However, it is a challenge for russian people not to watch these kremlin propagandists of violence and war, neo-Nazi ideology in general on the TV screen, when they zombify the population of their country every day with such powerful charisma, expression, and shouts. And TV viewers watch and watch again. And the worst thing is that these broadcasts are not banned either by the authorities or by public institutions. Goebbels's propaganda of Nazism in the thirties of the last century in Germany in comparison with the current kremlin propaganda is just a child's game. Even there, the representatives of the Aryan nation eventually believed that they were really blue bloods and a supernation. As they say, "don't believe your eyes". And it is no coincidence that today visual perception is associated with thinking. In our interpretation, sight is not a physiological process only, but a psychic, mental one.

The second is information. Only the information available to the individual, provided in accordance with his/her knowledge and life experience, is generalized. That is why the russian or even pro-russian TV viewer turned out to be very premorbid within this frame. Information is not limited to the source; it can come and continue in the process of further zombification. But in any case, the less available information, the lower the probability that when superimposing images, a solution will be able to stand out, which will turn out to be decisive. Here, the most important factor is awareness of information obtained as a result of superconscious processes. After all, in modern psychology, consciousness is considered in three modalities: as a process, as a stable functional system, and as ability.

Let's note once again – the superconscious in the psyche appears in the form of the HO images that are formed in the process of summarizing visual information. As a result

of such a generalization, complex non-verbal gestalts are formed, which can perform several roles in the context of the superconscious. Firstly, they can be factors of moral, cognitive, etc. patterns of behavior. Secondly, in the form of an unconscious HO image a person may get ideas not only about artistic or scientific creativity, but also worldviews. Thirdly, the formation of the HO images can be a part of thinking that provides an unconscious solution to creative, intellectual, or worldview problems. Fourthly, they can be one of the mechanisms of intuition. Other roles are possible too. Part of the roles are performed by the HO images already formed by the superconsciousness. Performing other roles requires that the image is formed in the process. The most static are the HO images that perform the function of a standard. Some authors assume the existence of certain non-verbal constructs in the psyche, which a person is oriented towards, is fully aware of, but cannot explain their essence.

In our research, we assumed that there are non-verbal gestalts for many complex phenomena. People use them in the process of thinking, when solving creative tasks, when choosing a life path, when determining the actions of their existence, but at the same time they are completely unaware of their meaning and consequences for themselves. The role of the visual sphere in the creation of creative thinking is great. According to the representatives of Gestalt psychology, any intellectual, vital, logical and any other task is the formation of a gestalt – a deep inner image [11, 12]. And the solution of a certain task occurs by transforming the gestalt. In practice, such a decision is carried out in three stages, the first stage – indicative activity, chaotic search for a solution, accumulation of information about the environment; the second stage is a pause, when information is collected, all possible options are considered, and a solution is not found, and then the process begins, which at first glance has the appearance of "freezing" of intellectual activity; the third stage is enlightenment.

Thus, during the period of information accumulation, a gestalt is formed, that is, a generalized image of the environment is created, in which all the seen pictures of all attempts to solve the task will be present. During the pause period, the gestalt is transformed so that the solution stands out as a figure from the background. Insight is the first moment of awareness of a solution found through Gestalt transformation (i.e., superconsciously). We believe that the gestalt formed in the process of orientational activity is a HO image. It can be assumed that such images are multi-meaningful and can unfold to the conscious part of the personality in different ways depending on their specific content and can highlight one meaning while others will remain in the shadows.

It should be noted that in our psyche there are many complex images that serve as standards that influence behavior, but are not realized by a person. It is worth paying attention to the following feature of the psyche: each of us uses only part of the information we perceive when solving immediate tasks, when communicating, etc. If every time a person turned to his/her consciousness, his/her activity would require much more time. However, to understand the information that was formed as a result of the generalization of a HO image is a separate task, and another task, no less difficult, is to convey it to other people.

To become conscious, these images must be connected to a system of words as meanings. Until this happens, many complex generalizations will be stored in the human psyche in the form of the HO images, even when the person does not have the corresponding definitions. In order to understand the HO images in our own psyche, we must connect them with a system of relevant concepts, that is, for some information to become conscious, it must line up in a system of concepts. Only in this way the superconscious will become available to our "I". But here another problem arises – a zombified person is not able to sufficiently realize the processes of his/her thinking.

Next is motivation. Nothing happens without motivation. It is motivation that ensures the formation of social behavior of a person. Summarizing this, we believe that motivation is the energy that ensures the formation of images of the third, fourth and even higher level from ordinary visual social image information. Images will be generalized, visual information will be superimposed and integrated as long as the person is eager to solve the task. This especially applies to the HO images which are actively formed at the same time, because they are related to cognitive, personal, and ideal tasks, that is, for which there is no natural motivation that comes from vital needs. Motivation can be of a social nature (putin and the zombified russian society say "should") or personal (a person motivates him/herself based on his/her own semantic attitudes). In any case, the formation and processing of visual information by the superconsciousness will take place as long as the motivation is active.

CONCLUSIONS

With the beginning of the russian full-scale attack on Ukraine, russian atrocities in Bucha, Mariupol and other cities and villages of the country, many Ukrainian citizens asked about what has happened to the russian society, the state authorities, who set the goal of destroying Ukraine as a state and all its inhabitants as a nation. Then Ukrainians have labelled the invaders and the authorities of Russia as non-humans. And this is a fair name for them. The fact is that these occupiers and their neo-Nazi leaders have a destroyed, distorted consciousness as a result of which they became incapable of realizing their thought processes. The consciousness of such persons gradually degrades towards animal thinking, the so-called proto-thinking. This is one direction to not

realizing one's intentions and actions at the level of both subconscious and partially conscious analysis of primary mental images (images of a first and second orders). The second direction is the role of superconscious processes, in particular mental images of a higher level, which also form the worldview positions of an individual in the process of viewing and listening to certain information, while remaining, at the same time, unconscious until a certain time. Together, these directions form a person's attitude to existing social and worldview problems.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Vitalii Shmarhun
National University of Life and Environmental Sciences of Ukraine,
19 Horikhuvatskyi shliakh st, 03041 Kyiv, Ukraine
e-mail: shmargun2012@ukr.net

ORCID AND CONTRIBUTIONSHIP

Vitalii Shmarhun: 0000-0001-9727-5083 A B D E F
Denys Shpak: 0000-0003-0545-6560 B E F
Lidiia Spivak: 0000-0002-1183-5682 B E F
Svitlana Shkil: 0000-0003-3859-816X B E F
Ihor Hoian: 0000-0003-2548-0488 B E F

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REVIEW ARTICLE

CONTENTS

Healthcare accessibility in the prison environment: Oral health

Jakub Fiegler-Rudol[1], Matuesz Migas[2], Maciej Budzik[3], Dawid Hochuł[1], Olga Odrzywolska[1], Patrycja Doroz[1], Kaja Zawadzka[2], Stanisław Niemczyk[4]

[1]FACULTY OF MEDICAL SCIENCES IN ZABRZE, MEDICAL UNIVERSITY OF SILESIA, ZABRZE, POLAND
[2]ST. LUKE HOSPITAL, TARNÓW, POLAND
[3]SPECIALIST HOSPITAL NAMED AFTER STEFAN ŻEROMSKI IN CRACOW, CRACOW, POLAND
[4]MUNCIPAL HOSPITAL NO.4 IN GLIWICE, GLIWICE, POLAND

ABSTRACT

This article aims to assess the importance of caring for oral health among prisoners, as an often-overlooked aspect of general health well-being in prisons. The incidence of oral disease among prisoners compared to the general population is much higher due to many factors such as limited access to dental care, unhealthy lifestyles and overcrowded cells. The research review confirms the need to increase access to dental care and promote hygiene awareness among inmates. Differences between genders and the impact of the length of the sentence on oral health are also pointed out.

KEY WORDS: epidemiology, oral health, prison

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INTRODUCTION

The oral health of prisoners is an important but often neglected component of overall health and well-being in prisons. Existing disparities between male and female prisoners, as well as the numerous factors contributing to dental problems, present healthcare systems with unique challenges in providing timely and dignified healthcare. Due to overcrowding, limited financial resources and increased risk of infectious diseases among prison populations, the impact of preventive activities on physical and mental health becomes particularly important. Most prisoners return to society after serving their sentence, which makes the care of oral health in prisons also important for the entire healthcare system [1-2].

AIM

The article aims to highlight the importance of oral health among prisoners, a population that often faces significant disparities in access to dental care. The authors emphasize the need for comprehensive planning of oral hygiene care in the prison environment, as well as the development of gender-specific oral care products to address the higher incidence of oral diseases among female prisoners.

MATERIALS AND METHODS

A systematic literature search was conducted in the PubMed, Embase, and Cochrane databases from inception to January 2024 to identify relevant studies. The search terms included "oral health", "prisoners", "dental care", "incidence", and related keywords. No language restrictions were applied. Studies were included if they reported on the oral health status, dental care behaviors, or interventions targeting oral health among prisoners. Studies focusing on the general population or other specific patient groups were excluded. Two reviewers independently screened titles, abstracts and full-text articles for eligibility. Disagreements were resolved by consensus or consultation with a third reviewer. Data extraction was performed by one reviewer and checked by a second reviewer. The following information was extracted: study design, country, sample size, participant characteristics, oral health outcomes, and key findings.

REVIEW AND DISCUSSION

ORAL HEALTH STATUS IN PRISONS

There is a higher incidence of oral diseases among prisoners (25.90%) compared to the general population (12.4%). Moreover, it was shown that female prisoners suffered from

diseases of the oral cavity and especially mucous membranes significantly more often (36.19%) compared to the general population (24.70%) [3]. Oral disorders affect ¼ of prisoners, which confirms the universality of this problem. A systematic review of 21 studies by Kumar et al. confirmed the higher incidence of oral diseases in the prison population compared to the rest of society, attributing it to inappropriate oral hygiene tools and insufficient dental knowledge and care among inmates [4]. The authors agree that the key actions to improve the health of prisoners are increasing access to a dentist and promoting hygiene awareness. In a broader perspective, health problems, including dental problems of prisoners, are considered not only a problem of the prison system, but also a social problem, given the eventual return of most of these people to society [5].

In the study by Yang et al. the incidence of oral disease in prisons was higher in women, which was attributed to hormonal differences affecting oral microbiota and salivary gland function. Recognizing gender differences in oral health, the study highlights the importance of developing gender-specific oral care products. Highlighting the social impact, it said it was essential to address oral health issues in prisons, emphasizing the need to include oral health services for prisoners in national health care to safeguard their well-being upon reintegration into society [6].

Healthcare systems should provide both timely and equitable dental services for prisoners, creating an opportunity for comprehensive planning of oral hygiene care in the prison environment. The incidence of dental caries among prisoners is much higher than in the general population (prevalence of 77%). This is consistent with previous research indicating the need to pay close attention to oral health problems. Differences in the advancement of caries lesions among prisoners were observed depending on age and gender, with the number of teeth removed due to caries and teeth with fillings increasing significantly with the age of prisoners. This was also higher among women. The severity of periodontal disease and caries was directly proportional to the length of imprisonment, which indicates the negative impact of long waiting periods for treatment and insufficient infrastructure in prisons on oral health [7-8]. Studies of people in contact with penitentiary institutions have shown that the following are significantly more common in the prisoner population [2]: high incidence of caries and periodontal diseases [9], ranging from 57% to 67% [10-11], poor oral health and incorrect eating habits [1], these factors contribute to the deterioration of the quality of life of inmates [12-13].

CHALLENGES IN PROVIDING DENTAL CARE TO PRISONERS

Currently, approximately 11 million people around the world are incarcerated in prisons every day [14]. Overcrowded prisons worsen health problems, and long-term isolation contributes to prisoners' poor oral health. The complex relationship between general health and oral diseases is due to the multifactorial nature of systemic diseases that directly or indirectly affect oral health. Factors such as smoking, alcohol, drug use and limited access to quality health care services contribute to the disastrous oral health of inmates. Eliminating inequalities in this area and promoting preventive measures in the primary health care system are crucial in mitigating the long-term consequences of oral diseases among prisoners, both during imprisonment and after release, which emphasizes the need for comprehensive dental care in prison conditions [15].

A study of the Minas Gerais prison population in Brazil analysed the self-assessment of the impact of oral health on the quality of life of female prisoners. The factors with the greatest influence included: psychological discomfort (50.5%), physical pain (40.4%) and unfavourable social situation (17.2%). The number of dental consultations, self-assessment of general health and ethnicity were also analysed. The study found significant associations between oral health and mental health, highlighting the prevalence of symptoms of anxiety (63.6%) and depression (42.4%) among female prisoners. The study highlighted the complex relationship between incarceration, mental health, and oral health, highlighting the need to develop comprehensive health policies in prison settings [16-17].

The patient himself is often a challenge for the dentist. Dental tools can be used as weapons or means of self-harm. Searching through medical waste, consulting with inmates, and checking the movement of patients in the clinic are typical procedures in prisons that contribute to limiting the possibility of admitting many patients per day. Arranging dental appointments for prisoners is like civilian practices, with the same fear of dental treatment for both groups of patients. There are also additional factors that hinder inmates' access to treatment, such as arrest warrants or a lack of staff supervising the patient during treatment. Prisoners, similarly, to the general population, most often refrain from dental treatment after being prescribed antibiotics that reduce pain or due to the prolonged waiting time for an appointment, which may intensify, especially in this group of patients, often suffering from psychiatric problems, aggressive behaviour resulting from impatience [18].

INITIATIVES AND PROGRAMS FOR PRISONERS' ORAL HEALTH

Strategies based on interviewing and encouraging dental check-ups result in positive motivational and behavioural changes. A motivational technique adapted to the conditions of inmates' perception may be a promising tool used to improve the oral health of prisoners. In a research Cascaes

et al . even a single motivational session brought positive effects, which is particularly important due to the transient nature of prisoners' stays and the resulting lack of possibility of long-term action [19].

In a cross-sectional study conducted in a Pakistani prison, researchers examined the dental care behaviours of 225 adult prisoners. In the study group, urban residents had a higher frequency of dental visits, while more than half of the prisoners had never visited a dentist. The main motivation for visiting the dentist was pain or discomfort in the mouth. Study participants from rural areas had statistically significantly fewer teeth (from 10-19 teeth) than inmates from urban areas who regularly had check-ups. Living in large cities and using dentures influenced regular visits. The study suggests the need to improve access to dental care in rural areas, as well as education on the importance of regular check-ups for incarcerated people [20- 21].

TELEMEDICINE AND TECHNOLOGICAL SOLUTIONS

The growing global prison population is accompanied by an increased incidence of health problems among prisoners compared to the general population. This includes a significant increase in mental health disorders - one in seven prisoners in Western countries suffers from mental illness. Moreover, infectious diseases are common in prisons, especially those caused by blood-borne viruses and sexually transmitted diseases [22]. Despite various service delivery models, access to timely health care in prisons remains a challenge, and factors such as organizational barriers and a shortage of medical staff contribute to limited access to health care while serving a sentence [23].

In France, prisoners in prisons are obliged to undergo dental examinations, but only half of them are willing to undergo regular preventive care. The possibility of potentially improving oral hygiene in prisons was investigated using telemedical consultations, during which dentists remotely assess patients' dental needs. The study, conducted in the form of an experiment, showed that in dental emergencies, the diagnosis made remotely by a dentist differs slightly from that made in person. Unfortunately, it should be emphasized that dental teleconsultation in cases of preventive care is characterized by a significant percentage of incorrect diagnoses, amounting to as much as 63%. France's overcrowded prison system faces challenges in providing adequate dental care, with only 52% of prisons offering required check-ups due to a shortage of dentists. Despite the high demand for dental consultations among inmates, there are no dentists willing to work in prisons. Numerous organizational problems result in limited access and failure to arrange visits to penitentiary facilities. It is recommended that oral health consultations be included in mandatory medical examinations for prisoners and telemedicine, in particular teleconsultations, could play a key role in addressing the challenges and improving dental care for prisoners, at least in the case of emergencies [24].

Telehealth, defined as the delivery of health services remotely using information and communication technologies, may address these issues, although evidence of its effectiveness with prisoners has not yet been assessed. Comparing the efficiency of teleconsultation and a conventional visit, research indicates long-term cost reduction in favour of telemedicine. Patient satisfaction with remote consultations is generally high, especially in psychiatry, dermatology, and multidisciplinary services [25]. Although patient satisfaction shows a positive impact on frontline telehealth in prisons, long-term follow-up and ongoing research are needed to evaluate clinical outcomes and cost-effectiveness. Successful implementation requires consideration of many factors, acceptance of teleconsultation among interested parties, and a comprehensive strategy that considers local factors and barriers [26].

HEALTH POLICY

Most governments do not see oral health as important. It was not until 2000 that Deputy Secretary of Health David Satcher recognized oral health challenges as a "silent epidemic" in the first-ever report on the subject [27]. Public health initiatives, such as Healthy People 2020, have not addressed the oral health needs of people in prisons. This is a significant problem because the approximately seven million Americans under prison jurisdiction face significant disparities in access to dental care [28-29]. Prisoners remain an "invisible" population in the system despite their right to health care. The authors emphasize the need to include oral health in clinical guidelines and improve the equipment infrastructure [30]. For the dental team to provide efficient and effective dental services in a prison setting, they must have a comprehensive knowledge of prison structures and processes. This requires effective communication and cooperation between prison managers, prison service and security staff [31-32]. It is recommended to involve dental hygienists and therapists working with prison health care staff when planning and conducting oral hygiene initiatives. It is essential to demonstrate professional standards through peer review, assessment, and clinical audit [33]. The role of public policy in shaping access to dental care is crucial and highlights the multi-faceted impact of decision-makers decisions on the quality of health care and the overall effectiveness of the system [34].

CONCLUSIONS

In prisons, oral health is an often-overlooked aspect of prisoners' overall health and well-being. The key factors contributing to the prevalence of dental problems in prisons are lifestyle, improper diet, stimulants, limited access to dental treatment and low motivation of this group of patients for control and prevention. Challenges related to providing adequate dental care in prisons include mainly prison overcrowding and limited human resources among medical staff who are reluctant to work in penitentiary facilities. Programs aimed at improving prisoners' oral health, including motivational interviews, promoting oral hygiene and preventive care, may help improve the poor situation. Telemedicine can be considered as a potential way to partially solve the problems related to difficult access to health care in prison conditions. Health policy changes are key elements in improving the oral health of prisoners, highlighting the need for a comprehensive approach involving policymakers, health professionals and community organizations to achieve lasting change. It is essential to recognize and address the oral health needs of this often overlooked and excluded population.

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CORRESPONDING AUTHOR

Jakub Fiegler-Rudol
Faculty of Medical Sciences in Zabrze,
Medical University of Silesia, Poland
e-mail: jakub.fiegler@gmail.com

ORCID AND CONTRIBUTIONSHIP

Jakub Fiegler-Rudol: 0009-0009-3173-9389 A B D
Matuesz Migas: 0009-0001-0274-7832 D E
Maciej Budzik: 0009-0009-1071-5089 D
Dawid Hochuł: 0009-0008-5894-4959 A
Olga Odrzywolska: 0009-0003-2674-8447 C D E
Patrycja Doroz: 0009-0004-9266-0360 D
Kaja Zawadzka: 0009-0001-8812-6083 E F
Stanisław Niemczyk: 0000-0001-5236-2298 E F

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CLINICAL CASE

CONTENTS

Creutzfeldt – Jakob disease: clinical cases

Khrystyna Yuskiv[1], Anzhelika Payenok[2], Radu Protsiuk[3], Galyna Pryshliak[4]
[1]SECOND MEDICAL UNION, 5TH HOSPITAL, LVIV, UKRAINE
[2]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE
[3]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[4]ST. PANTELEIMON HOSPITAL, LVIV, UKRAINE

ABSTRACT
A 68 y.o. woman delivered to the emergency department with severe speech impairment in a somnolent state - 13 points Glasgow Coma Scale. Her relatives described a clinical manifestation: rapidly progressive dementia, visual disturbances, abnormal gait and coordination, retrograde amnesia. A 67 y. o. man delivered to the emergency department with headache, vertigo, abnormal gait and coordination, progressive extremities weakness, disorientation, memory and concentration impairment. His relatives told about extremely rapidly progression of symptoms during last three months. First clinical case presents a Heidenhain variant, second case – Brownell - Oppenheimer variant.

KEY WORDS: rapidly progressive dementia, prion disease, diagnostic criteria in vivo

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INTRODUCTION

Creutzfeldt - Jakob disease (CJD) is a transmissible spongiform encephalopathy that manifests as a rapidly progressive dementia. There are four types of CJD: sporadic, variant, familial, iatrogenic. It is not very common disease, but nowadays the molecular basis of prion propagation and the pathogenesis of illness are well understood. All human prion diseases have similar pathomorphological features – spongiform degeneration of the gray matter, gliosis, neuronal loss, and deposits of insoluble prion protein (PrP^{Sc}) [1]. There are strict and recognizable clinical features and imaging patterns on magnetic resonance (MR) imaging helping a neurologist to be confident in diagnosis. In this article, we described two clinical cases of CJD observed in St. Panteleimon Hospital during 2023 year.

CASE REPORT

1 CASE

A 68 y.o. woman delivered to the emergency department with severe speech impairment in a somnolent state - 13 points Glasgow Coma Scale. Her relatives described a clinical manifestation: rapidly progressive dementia, visual disturbances, abnormal gait and coordination, retrograde amnesia. According to the patient`s history, she had coronary artery disease, II stage of an essential arterial hypertension, and also complicated professional anamnesis – patient worked with a fresh meat of domestic animals. General examination was performed in emergency department: BP 160/100 mm Hg, heart tones were rhythmic.

Neurological examination showed horizontal nystagmus with a right side gaze fixation, facial asymmetry, left side tongue deviation, extrapyramidal signs, tendon and periosteal reflexes D>S, subcortical signs. Active movements in upper and lower extremities were restricted due to muscle rigidity. The patient had abnormal bilateral foot signs – Babinski, Strumpel. Romberg`s test wasn`t performed due to her somnolence.

MRI findings: cortical hyperintensity in right frontal, temporal and occipital lobes (DWI), hyperintensity in right nucleus caudatus, putamen and thalamus (fig. 1). Cerebral microangiopathy signs also were presented. EEG investigation demonstrated periodic sharp wave complexes in the left parieto-temporal lobe.

2 CASE

A 67 y. o. man delivered to the emergency department with headache, vertigo, abnormal gait and coordination, progressive extremities weakness, disorientation, memory and concentration impairment. His relatives told about extremely rapidly progression of symptoms during last

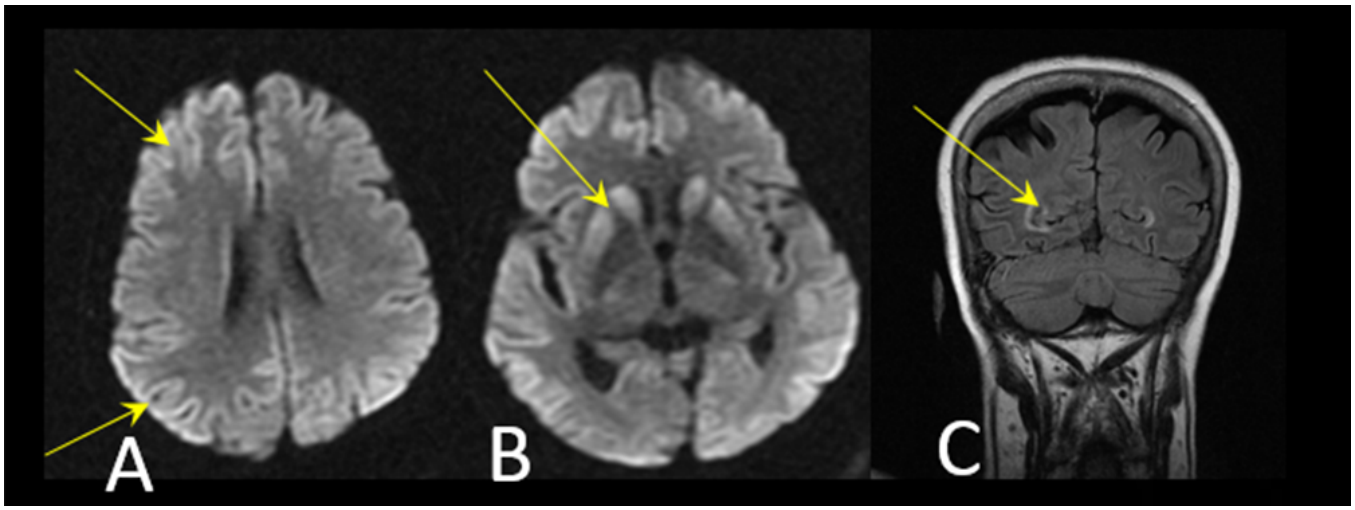

Fig. 1. MRI findings: A - cortical hyperintensity (DWI), B and C - hyperintensity in right nucleus caudatus, putamen and thalamus (DWI, FLAIR).

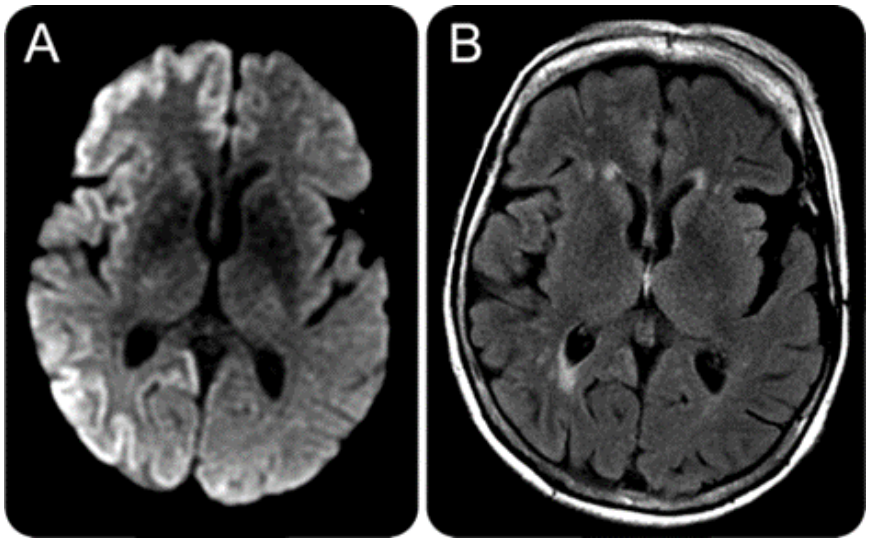

Fig. 2. The axial diffusion-weighted image (A) showed restricted diffusion throughout the cortical ribbon of the right hemisphere. There was corresponding decreased signal on the apparent diffusion coefficient map. The changes were not easily seen on the fluid-attenuated inversion recovery sequence (B).

three months. A patient had coronary artery disease, II stage of an essential arterial hypertension, and also complicated professional anamnesis – he worked with a fresh meat of domestic animals. General examination was performed in emergency department: BP 160/90 mm Hg, heart tones were rhythmic.

Neurological status: conscious - 15 points Glasgow Coma Scale, periodic time and place disorientation, horizontal nystagmus, cerebellar ataxia, left side pyramidal signs. The Romberg`s test showed ataxia. The patient performed a finger-to-nose test unsatisfactory. Tendon and periosteal reflexes D=S. Active movements in upper and lower extremities without any restrictions.

MRI findings: cortical hyperintensity in frontal, temporal, parietal and occipital lobes bilaterally, mostly on the left hemisphere without damage of basal ganglia (DWI). A small vessel disease and degenerative cortical changes were detected in T2 scans.

Diagnosis of CJD remains a challenge because of the large variability of the clinical symptoms especially in its early stages of disease. The Centers for Disease Control and Prevention (CDC) listed recognizable clinical features and paraclinical tests to supplement the replicable diagnostic criteria in vivo (table 1).

There is a high diagnostic accuracy of CSF real-time quaking-induced conversion but identified inflammatory brain disease as a potential source of (rare) false-positive results, indicating thorough consideration of this condition in the differential diagnosis of Creutzfeldt-Jakob disease [3].

Nowadays there are typical MR imaging patterns in sporadic CJD such as cortical and basal ganglia involvement with hyperintensity at DWI in the insula and cingulate (limbic lobe), involvement of the superior frontal gyri and the cortical areas near the midline [4]. The perirolandic area is usually spared. In 2020, a study investigating a large cohort with 770 patients with definite sporadic Creutzfeldt - Jakob disease applied an improved diagnostic index showing 92% sensitivity and 97% specifici [5].

Table 1. The CDC`s diagnostic criteria of CJD [2].

Diagnosis	Definite	Probable	Possible
Sporadic CJD	Diagnosed by standard neuropathological techniques; and/or immunocytochemically; and/or Western blot confirmed protease-resistant PrP; and/or presence of scrapie-associated fibrils.	Neuropsychiatric disorder plus positive RT-QuIC in cerebrospinal fluid (CSF) or other tissues OR Rapidly progressive dementia; and at least two out of the following four clinical features: myoclonus; visual or cerebellar signs; pyramidal/extrapyramidal signs; akinetic mutism. AND a positive result on at least one of the following laboratory tests: a typical EEG (periodic sharp wave complexes) during an illness of any duration; a positive 14-3-3 CSF assay in patients with a disease duration of less than 2 years; High signal in caudate/putamen on magnetic resonance imaging (MRI) brain scan or at least two cortical regions (temporal, parietal, occipital) either on diffusion-weighted imaging (DWI) or fluid attenuated inversion recovery (FLAIR). AND without routine investigations indicating an alternative diagnosis.	Progressive dementia; and at least two out of the following four clinical features: myoclonus; visual or cerebellar signs; pyramidal/ extrapyramidal signs; akinetic mutism AND the absence of a positive result for any of the four tests above that would classify a case as "probable" AND duration of illness less than two years AND without routine investigations indicating an alternative diagnosis.
Iatrogenic CJD	Progressive cerebellar syndrome in a recipient of human cadaveric-derived pituitary hormone; or sporadic CJD with a recognized exposure risk, e.g., antecedent neurosurgery with dura mater implantation.		
Familial CJD	Definite or probable CJD plus definite or probable CJD in a first degree relative; and/or Neuropsychiatric disorder plus disease-specific PrP gene mutation.		

Table 2. Clinical subtypes of CJD [6]

Features	Subtypes		
	Heidenhain	**Brownell-Oppenheimer**	**Stern**
Symptoms and signs at onset	Isolated visual symptoms: poor vision; disturbed perception of colors or structures; optical distortions and hallucinations. All without any ocular disease. May also occur in genetic CJD.	Isolated cerebellar ataxia	Presenile dementia and sleep disturbances: inability to initiate and maintain sleep; frequent arousals; enacted dreams. Younger age at onset than the other groups.
Molecular genetic correlation	Most are MM-MV1 type. Rare cases due to MM 2C and MM 2C+1.	VV2	MM2 C*
Paraclinical tests	EEG: PSWCs. CSF analysis: t-tau and 14-3-3 protein levels increased.	EEG: absence of PSWCs. CSF analysis: 14-3-3 protein levels not elevated.	EEG: absence of PSWCs CSF analysis: 14-3-3 protein levels not elevated in most cases.
Imaging	80% show occipitoparietal hyperintensity at DWI or FLAIR	Cerebellar atrophy. Increased ADC ± BG and cortical findings at DWI	Hypoperfusion or hypometabolism at SPECT and PET No signal intensity alterations at DWI

Note: BG = basal ganglia, C = cortical, DWI = diffusion-weighted imaging, PET = positron emission tomography, PSWCs = periodic sharp wave complexes, SPECT = single photon emission computed tomography.
* This variant (MM2 thalamic) is virtually indistinguishable from fatal familial insomnia.

It is important to remember about mimicking disorders such as severe hypoxic ischemic encephalopathy, hypoglycaemia, autoimmune-mediated encephalopathy, encephalitis, hyperammonemia, mitochondrial disorders, extrapontine osmotic demyelination, thalamic abnormalities [6]. Their MR imaging features can mimic CJD imaiging patterns, especially a cortical ribbon sign (fig. 2), [7].

There are three main clinical subtypes of CJD: Heidenhain, Brownell - Oppenheimer, Stern (table 2), [6].

CONCLUSIONS

Described clinical cases correspond to the criteria of possible CJD according to CDC's Diagnostic Criteria for Creutzfeldt - Jakob Disease (2018). First clinical case presents a Heidenhain variant, second case - Brownell-Oppenheimer variant. The most recommended approaches to in vivo diagnosis of CJD are strict application of diagnostic criteria, careful interpretation of neurovisualization, EEG.

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CORRESPONDING AUTHOR

Khrystyna Yuskiv
5th Hospital
26 Konovaltsia st., 79013 Lviv, Ukraine
e-mail: christiayu3@gmail.com

ORCID AND CONTRIBUTIONSHIP

Khrystyna Yuskiv: 0009-0001-4364-1508 C D
Anzhelika Payenok: 0000-0003-0531-751X F
Radu Protsiuk: 0000-0002-8434-8949 B E
Galyna Pryshliak: 0009-0006-4490-5524 A

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SHORT COMMUNICATION

CONTENTS

Challenges and opportunities in the Polish healthcare sector from the implementation of telemedicine solutions in the field of gerontology and geriatric medicine: own experience

Beata Jankowska-Polańska[1,2], Bartosz Sapilak[3], Magdalena Kałuska[4], Tomasz Mazurek[5], Jolanta Oen[6], Artur Janusz[6], Wojciech Tański[2,7]

[1]CENTRE FOR RESEARCH AND INNOVATION, 4TH MILITARY CLINICAL HOSPITAL IN WROCLAW, WROCLAW, POLAND
[2]FACULTY OF MEDICINE, WROCLAW UNIVERSITY OF SCIENCE AND TECHNOLOGY, WROCLAW, POLAND
[3]DEPARTMENT OF FAMILY MEDICINE, WROCLAW MEDICAL UNIVERSITY, WROCLAW, POLAND
[4]DEPARTMENT OF FUNDS ACQUISITION, 4TH MILITARY CLINICAL HOSPITAL IN WROCLAW, POLAND
[5]CLINICAL RESEARCH SUPPORT CENTRE, 4TH MILITARY CLINICAL HOSPITAL IN WROCLAW, WROCLAW, POLAND
[6]DEPARTMENT OF TELEMEDICINE, HORIZON GROUP AS IN NORWAY, NORWAY
[7]DEPARTMENT OF INTERNAL MEDICINE, 4TH MILITARY CLINICAL HOSPITAL IN WROCLAW, WROCLAW, POLAND

ABSTRACT

Aim: To analyze and summarize the implementation of telemedical solutions in geriatrics and gerontology within the Polish healthcare sector, aiming to develop innovative strategies for improving elderly care through telemedical technologies.

Materials and Methods: An interdisciplinary pilot project in geriatrics was implemented, focusing on health, organizational, and technological areas. The project involved continuous monitoring of health parameters, remote consultations, and the use of telemedical devices and platforms. Key data collection tools included digital clinimetric outcomes from the FRA-MNA-SARC model, with data transmitted to a telemedical platform.

Results: The pilot project demonstrated significant positive outcomes for senior participants. Continuous monitoring of health parameters allowed for early detection and timely intervention, leading to noticeable improvements in chronic disease management. This proactive approach reduced emergency hospital visits and enhanced overall health stability. The medication adherence support system, with automated reminders, ensured patients took their medications as prescribed, resulting in improved compliance and health outcomes. Telemedical solutions efficiently reduced the need for frequent in-person visits, allowing healthcare providers to monitor progress and adjust therapies in real-time. The project also effectively engaged patients and caregivers, increasing confidence in health management and providing valuable support and real-time information.

Conclusions: Implementing telemedical solutions in geriatrics within the Polish healthcare sector shows significant potential to improve elderly care. Telemedicine can effectively support chronic disease management, enhance seniors' quality of life through continuous health monitoring, and provide a practical framework for personalized and efficient healthcare delivery.

KEY WORDS: telemedicine, e-health, geriatrics, frailty, opportunities, challenges

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INTRODUCTION

Through the application of telemedical solutions, continuous monitoring of key health parameters becomes feasible, contributing to the effective management of chronic conditions in the elderly [1]. This transformative potential underscores the importance of integrating telemedicine into healthcare strategies, aiming to enhance the quality of life for seniors by addressing their specific healthcare needs [2]. Its ability to overcome geographical barriers and facilitate remote consultations makes telemedicine particularly beneficial for seniors who may face challenges in accessing traditional healthcare facilities [3]. The ongoing evolution of telemedical technologies holds the promise of further innovations, potentially revolutionizing geriatric care by providing timely interventions, preventive measures, and tailored healthcare plans that align with the unique health requirements of the aging demographic [4,5]. As telemedicine continues to advance, it is anticipated to play a pivotal role in

optimizing healthcare delivery and improving the overall well-being of the elderly [6,7].

Telemedicine revolutionizes the monitoring of senior health, enabling continuous tracking of key parameters without the need for their physical presence in a medical facility [8,9]. With advanced medical devices and mobile applications, blood pressure, glucose levels, pulse, and oxygen saturation can be monitored [10]. This not only allows for early detection of potential health issues but also facilitates a prompt response, which is crucial for providing comprehensive care to seniors. Seniors can now consult with doctors without the need to travel, using video conferencing and telemedical platforms. This is particularly important for individuals with limited mobility, as it provides easy access to specialized healthcare from the comfort of their homes [11].

The pilot project focused on patients with chronic conditions such as diabetes and heart disease. By integrating telemedicine, we provided continuous health monitoring through devices that automatically transmitted data to a central care platform [12, 13]. The automatic transmission of data from monitors to remote care platforms enables doctors to track progress and adjust therapies in real-time [14]. For example, one patient with a history of hypertension experienced significant improvement in managing their condition due to real-time monitoring and timely adjustments in their treatment plan based on data received from their home blood pressure monitor. This integration not only optimized disease management but also reduced the need for frequent in-person visits, thereby improving the overall quality of life for our senior participants [15,16].

Finally, telemedicine is becoming a key tool in ensuring the safety of seniors, especially those living independently [17]. Monitoring systems respond to sudden events, such as falls, automatically notifying emergency services or caregivers. This ensures a rapid response in case of emergencies, increasing the sense of security for seniors and their families [18]. Access to telemedicine stimulates seniors' autonomy, allowing them to actively monitor and manage their own health. This tool not only improves the quality of life through better healthcare but also promotes greater awareness and health education among the elderly, fostering healthier habits and self-care [19, 20].

In the face of dynamic challenges related to the care of the senior population, implementing modern solutions becomes a way to enhance geriatric care [21, 22]. This article summarizes a pilot project aimed at implementing and testing innovative telemedical solutions in the field of geriatrics. By focusing on the practical implementation of telemedical solutions, this project not only explores the potential of modern technologies but also emphasizes the real benefits they can bring to patients, medical staff, and the healthcare system as a whole. Encompassing areas such as the analysis of geriatric needs, the selection of appropriate technologies, staff training, effectiveness testing, and acceptance evaluation, this project sheds light on the comprehensive process of adapting telemedicine in geriatrics [23].

In this article, a closer look will be taken at the key stages of the project, with the obtained results, conclusions, and prospects for further development being analyzed. The introduction of telemedicine into the field of geriatrics is not only opening up new diagnostic and therapeutic possibilities but is also raising the question of how-to best tailor these modern tools to the unique needs of seniors. Acting as a bridge between traditional care and innovative technologies, this project contributes to the creation of more accessible, effective, and personalized geriatric care.

AIM

The aim of this article is to analyze and summarize the implementation of telemedical solutions in the context of geriatrics and gerontology in the Polish healthcare sector. The article seeks to identify challenges and opportunities associated with the implementation of telemedical technologies in the care of elderly individuals. Through the analysis of experiences and results from pilot projects and existing initiatives, this article aims to provide insights into the effectiveness, acceptance, and potential benefits of implementing telemedicine in geriatric healthcare in Poland. The ultimate goal of the article is to contribute to the development of innovative strategies for improving care for the elderly through the use of telemedical technologies.

MATERIALS AND METHODS

To achieve the goals set by this article, an interdisciplinary and comprehensive research framework was applied. Below, the steps and data collection strategy are presented, which enabled the achievement of the goal of analyzing the implementation of telemedical solutions in geriatrics and gerontology in the Polish healthcare sector. For each project, the goal, scope, applied telemedical technologies, and collected data were thoroughly analyzed. This information served as a starting point for identifying key issues related to the implementation of telemedicine in the care of elderly individuals. The effectiveness of monitoring the health of seniors was assessed, clinical

benefits were identified, and potential challenges related to the interpretation and management of health data within geriatric healthcare were evaluated. The level of acceptance of telemedical technologies by older individuals, understanding their experiences, and expectations regarding modern e-health solutions in healthcare were identified. The final summary of the materials collected within the described methodology allowed for a holistic view of the implementation of telemedicine in geriatric healthcare in Poland using the discussed pilot project as an example.

An attempt was made to identify challenges and draw conclusions to contribute to the development of innovative strategies for improving care for the elderly. The implementation of the project was summarized in the health, organizational, and technological areas. Key indicators from the pilot implementation were also outlined in relation to the assumptions of the project proposal within the Polish healthcare system.

The telemedical care project for patients with geriatric syndromes, focusing on frailty, sarcopenia, and malnutrition, is a response to existing challenges in healthcare for the elderly. The presented model provides a holistic range of home and outpatient care services, focused on three key geriatric areas. This project was created to overcome current barriers and meet the needs of patients. Due to multiple complications, these patients face longer waiting times. This, in turn, leads to situations where patients end up in the hospital in critical conditions, not only worsening their health but also negatively affecting their independence, life expectancy, and the possibility of effectively treating other diseases. The telemedical pilot aims to shorten these waiting times and improve healthcare accessibility for this patient group, aiming to increase the efficiency and quality of provided services [24].

The main goal of the project is to increase access to telemedical healthcare services for 520 individuals, including at least 260 women, aged over 60 years who have provided written informed consent to participate. At least 10% of the beneficiaries are intended to come from excluded areas, such as counties with per capita income below the national average, particularly rural areas distant from major cities. The plan includes conducting at least 520 screening examinations, and in cases of malnutrition, sarcopenia, and frailty syndrome, improving the prognosis for 80% of these patients during the pilot. The detailed objectives of the project included [24]:

- Enhancing independence and a sense of health security among the 60+ population, focusing on those affected by malnutrition, sarcopenia, and frailty syndrome, regardless of income or place of residence
- Eliminating economic and geographical barriers to ensure equal access to healthcare services
- Verifying the effectiveness of the proposed model for precise and efficient screening in the population
- Identifying individuals with malnutrition, sarcopenia, and frailty syndrome and implementing actions to improve their health
- Ensuring close collaboration between the Hospital, Primary Healthcare (PHC), and Ambulatory Specialized Centers (ASC), utilizing telemedicine tools efficiently
- Optimizing healthcare services for the 60+ population, emphasizing screening activities, secondary prevention, and health education
- Engaging PHC physicians as primary service providers for individuals identified with these conditions, using specialists (geriatricians) only when advanced support is needed
- Fully utilizing telemedical solutions to engage patients and caregivers in health care, rehabilitation, and treatment processes.

RESULTS AND DISCUSSION

HEALTHCARE FIELD

The summary encompasses an assessment of the project's impact on the health domain, focusing on clinical and health outcomes. The effectiveness of telemedicine in improving diagnosis, monitoring, and the efficiency of treatment in older individuals was analyzed. Potential health benefits for patients, such as improved quality of life, reduced waiting time for medical assistance, and a decrease in hospitalizations, were also considered.

The project focused on the application of three simple screening scales – MNA, SARC-F, and FRAIL – aimed at identifying patients with frailty syndrome, sarcopenia, or malnutrition. Within the project, the predominant form of patient interaction with the geriatrician was through teleconsultations, enabling effective health monitoring and consultations with the primary care physician – geriatrician. The project's goal included identifying patients with frailty syndrome, sarcopenia, or malnutrition, allowing for early identification and referral to appropriate remedial actions. The coordination and utilization of patient information were managed by a team consisting of primary care physicians, geriatric specialists, and community nurses. Data were collected and analyzed continuously, and in the case of detected irregularities, appropriate medical actions were taken. Data on blood pressure, glucose levels, pulse, oxygen saturation, and other vital health parameters were collected. Threshold

data points were established based on individual treatment plans, and any deviations from the norm were promptly reported to the medical team. Scales such as MNA, SARC-F, and FRAIL were selected due to their widespread use and effectiveness in identifying issues related to malnutrition, sarcopenia, and frailty syndrome. The selection of these scales was based on consultations with experts in the field of geriatrics.

The telemedical project encountered significant bureaucracy, significantly impeding its planned implementation. The overarching principle seemed to be the necessity of satisfying the Norwegian Partner and officials from the Ministry of Health (MZ), unfortunately leading to a lack of understanding of the realities and a willingness to cooperate. A key issue was the failure to establish and separate the organizational-administrative team from the medical team, resulting in "marginalization" and a lack of proper communication. This, in turn, generated uncertainty and demotivation within the medical team. Too many ineffective meetings without tangible progress contributed to narrowing down the actual medical activities to just a few months. Additionally, this situation prompted some partners to withdraw from participating in the project or limit the involvement of others. Ultimately, the lack of proper organization, bureaucracy, and inefficient meetings accumulated, contributing to the inability to fully realize the telemedical project in line with its original assumptions.

Based on experiences from the current project, a key insight is the necessity of separating, acquainting, and integrating the medical team. Initial training and a practical discussion of recruitment principles and the use of research tools are essential, as well as establishing effective communication channels among team members. The project encountered a "non-random" selection of patients, involving "active and fit" seniors affiliated with associations and universities for the elderly. While this expedited the recruitment process, it significantly influenced the final project outcomes. Challenges arose in reaching seniors with significant health problems and convincing them to participate in the program. The lack of practical means to ensure effective participation in the telemedical project for this group resulted in the project mainly encompassing the upper quartile of individuals with illnesses. Involving such patients would require assigning a social worker present during teleconsultations, ensuring full cognitive functions and understanding of questions by the patient. Despite recruiting patients with higher cognitive abilities, most of them reported difficulties with handling multimedia tools, leading to a predominance of phone consultations. These findings emphasize the need for a more equitable recruitment process and adapting telemedical tools to the diverse needs of patient groups, considering their technological proficiency.

The project experienced an inadequate selection of research tools, with only the MNA scale proving effective in accurately describing patients' issues. Tools such as the SARC-F scale and the FRAIL questionnaire proved inappropriate despite their ease of use, characterized by low specificity and discriminatory value. Evaluating research tools in the context of telemedical studies is challenging; however, it may be necessary to use more comprehensive scales, possibly incorporating the physician's functional assessment of the patient. Such a process would require staff training and conducting preliminary pilot studies. It is worth noting that despite advanced telemedicine, nothing can replace a thorough physical examination by a geriatrician or trained internist. Hence, careful consideration is needed when selecting research tools for telemedical projects, emphasizing effectiveness, specificity, and adaptation to the characteristics of the studied patient group.

ORGANIZATIONAL FIELD

The focus was on the project's impact on healthcare organization, identifying changes in the organizational structure and workflows of medical staff. The adaptation of administrative and logistical systems for the implementation of telemedicine was also analyzed, paying attention to potential time and resource savings.

According to the opinions of project participants and partners, the invaluable role of Team 4.WSK was a key element in the success of the telemedical project for seniors, serving as the foundation for its resilience and success. Without the commitment and perseverance of this team, the project could have faced numerous difficulties and even failed. During the project, it was possible to recruit and preliminarily examine 500 seniors (295 women, 205 men). However, difficulties arose in maintaining some of them in the project already during the stage of contacting patients and scheduling teleconsultation appointments. Direct communication with a geriatrician proved to be more effective, emphasizing the importance of direct communication in healthcare for seniors. Sixty-one geriatric consultations were conducted, with fifty-nine of them being realized. It is worth noting the lack of identification of individuals with malnutrition, although several patients were on the verge of being diagnosed with frailty syndrome. Despite some difficulties, the

project proved to be a significant success thanks to the determination of Team 4.WSK and effective efforts in recruitment and geriatric consultations.

In summary, the organization of future projects should commence at least 6-12 months before the planned start of recruitment. During this period, attention should be given to detailed training, meeting schedules, and the development of clear collaboration principles. Ensuring proper communication, understanding project goals, and coordinating the actions of different teams are crucial elements for the effective implementation of telemedical projects in the future.

The telemedical project faced several challenges that impacted its course and outcomes. Some patients withdrew after the initial recruitment stage, affecting the final composition of the studied group. Additionally, patient qualification for geriatric consultations was noted despite not meeting the criteria. The projection of late-stage COVID pandemic symptoms and the "Polish mentality," contributing to frequent fatigue among examined patients, significantly influenced the credibility of responses and the discriminatory power of the applied scales. As a result, not a single case of malnutrition was identified, and features of frailty syndrome often stemmed from underlying conditions such as a history of stroke or oncological diseases. Conclusions drawn from these challenges suggest that the project must flexibly adapt to changing conditions, considering the specificity of patients and their surrounding context. The need for redefining recruitment criteria and meticulous monitoring of the impact of external variables on project outcomes are key elements in planning and implementing telemedical research.

The telemedical project has achieved significant results, including the examination of 500 individuals, indicating the effectiveness of recruitment efforts. Acquiring organizational know-how and medical experience constitutes valuable capital for the future. The option to commission laboratory tests was an attractive feature for patients, suggesting potential development in this area. Access to geriatric consultations, even for individuals outside the initial project criteria, proved to be an additional advantage. The project activated seniors and provided material for further research and reflection, establishing a solid foundation for future initiatives.

TECHNOLOGICAL FIELD

In the project, several forms of communication were used, including mobile applications and telemedicine platform AURERO (Medily Ltd., Łódź, Poland), and mobile health applications compatible with monitoring devices. Seniors used these tools to monitor health parameters and communicate with medical staff. Before the pilot began, training sessions were conducted for seniors, covering the operation of applications, measuring devices, and telemedicine platforms. These training sessions were led by telemedicine specialists and community nurses, who later provided technical support. Patients reported technical issues related to the operation of devices and difficulties accessing the internet. Some patients also had concerns about privacy and data security. All reports were addressed promptly, and technical issues were resolved by a dedicated support team.

As part of the technological assessment of the project, a comprehensive analysis of aspects related to both telemedical infrastructure and the tools and platforms used was conducted. The focus was on evaluating the integration level of new technologies with existing systems, paying special attention to identifying potential technical challenges while indicating development opportunities and scalability of the applied solutions. The analysis of telemedical infrastructure included an assessment of its readiness to handle project requirements, with an emphasis on stability, reliability, and throughput. Regarding the tools and platforms used, their functionalities, interfaces, and compliance with current industry standards were evaluated.

Additionally, the degree of integration of new technologies with existing medical or information systems was considered, identifying potential pain points and the need for adjustment. The analysis also focused on the development possibilities and scalability of the applied solutions, with the prospect of potential project scope expansion or adaptation to new conditions in mind. The entire technological assessment process aimed to ensure that the telemedical infrastructure and tools used in the project are tailored to its specifics while allowing for potential modifications in line with project development or emerging technological trends (Table 1).

PROJECT INDICATORS

Within the project aiming to implement modern solutions in the healthcare sector, a key element is the systematic monitoring of progress and the effectiveness of activities. In this context, the project's key indicators play a crucial role, serving as measurable criteria for achievements and the efficiency of implemented actions. Monitoring these indicators is essential for evaluating the project's effectiveness and enables informed management decision-making.

Table 1. Summary of the issues related to the implementation of the pilot project, along with organizational and formal difficulties

Category	Problems
Technological challenges	Integration of various telemedicine systems and measurement devices was more complex than anticipated Compatibility issues between mobile applications and medical devices. Need to ensure stable internet connectivity for seniors, especially in rural areas
Training and adaptation of seniors	High level of unfamiliarity with new technologies among seniors. Need for multiple training sessions and provision of technical support. Challenges with motivating and engaging some participants in regular use of technology
Organizational challenges	Coordination of activities between different healthcare units (primary care physicians, geriatric specialists, community nurses) Problems with scheduling visits and monitoring patients Challenges related to data management and patient privacy protection
Formal challenges	Requirement to obtain appropriate permissions and consents for the use of new technologies in healthcare Regulations concerning data protection (GDPR) and ensuring compliance with legal requirements Issues with funding and resource allocation for the purchase and maintenance of telemedicine equipment
Technical issues	Unreliability and failure of some medical devices Difficulties in managing remote technical support for patients Problems with maintaining and updating telemedicine software

The pilot project demonstrated significant positive outcomes and impacts on the health and well-being of the senior participants, as well as on the efficiency of the healthcare delivery system. The implementation of telemedical solutions provided a practical framework for continuous health monitoring, effective management of chronic conditions, and enhanced medication adherence among the elderly population.

HEALTH OUTCOMES

The continuous monitoring of health parameters such as blood pressure, glucose levels, pulse, and oxygen saturation allowed for early detection and timely intervention in potential health issues. This proactive approach led to a noticeable improvement in the management of chronic diseases. For example, a participant with fluctuating glucose levels received timely alerts and medical advice, preventing a potential diabetic crisis. The overall health stability of participants was maintained and, in some cases, significantly improved, as evidenced by the reduction in emergency hospital visits.

MEDICATION ADHERENCE

The telemedical applications included a medication adherence support system that played a crucial role in maintaining proper medication habits among the participants. Automated reminders ensured that patients took their medications as prescribed, which resulted in improved compliance and overall health outcomes. Real-time updates to caregivers and healthcare professionals allowed for prompt intervention in cases of non-adherence, further enhancing the effectiveness of treatment plans.

EFFICIENCY OF HEALTHCARE DELIVERY

The project also highlighted the efficiency of telemedical solutions in reducing the need for frequent in-person visits. The automatic transmission of health data to remote care platforms enabled healthcare providers to monitor patients' progress and adjust therapies in real-time. This not only optimized disease management but also alleviated the burden on traditional healthcare facilities. For instance, the number of healthcare services provided with the assistance of purchased modern equipment reached 1299, which is 83% of the target value, indicating a high utilization rate of telemedical tools.

PATIENT AND CAREGIVER ENGAGEMENT

The project successfully engaged patients and caregivers in the healthcare process. The ease of use of telemedical devices and applications facilitated greater involvement in health management. Patients reported increased confidence in managing their health conditions, and caregivers benefited from the support and real-time information provided by the telemedical systems. The satisfaction rate among beneficiaries was high, with 96% of participants expressing satisfaction with the services received through new e-health methods (Table 2).

PRACTICAL IMPLICATIONS

The implementation of telemedical solutions in the care of elderly individuals can bring several practical benefits. One of the key implications is the ability to continuously monitor the health of seniors, enabling a prompt response to changes in their health conditions. This, in turn, can lead to more effective management of

Table 2. Key indicators of the geriatric project

Indicator	Achieved Value (n)	Target Value (n)	Performance Indicator (%)
Number of beneficiaries (female)	294	260	113%
Number of beneficiaries (male)	206	260	79%
Number of individuals expressing satisfaction with services received through new e-health methods according to the implemented model	500	520	96%
Number of healthcare services provided with the assistance of purchased modern equipment	1299	1560	83%
Number of primary healthcare units covered by telemedical service	9	9	100%
Number of organizations (primary healthcare units, foreign partners, patient organizations, domestic partners) collaborating with the Leader in the implementation and validation of the project	12	12	100%
Territorial scope of the project: Number of counties whose residents participate in the project	4	4	100%
Experience of the Applicant and Partners: Number of entities with experience in implementing at least one project in the field of e-health or telemedicine	5	5	100%
Project management: Number of entities that will apply PRINCE2 project management methodology and an approach in line with the principles of equality management and equal opportunities policy in project management	12	12	100%
Number of patients diagnosed with geriatric conditions: sarcopenia, malnutrition, frailty syndrome based on online consultations and questionnaires conducted with the patient	12	260	5%
Increase in the level of knowledge about using medical services online among project participants	105	520	20%
Increase in the level of knowledge about geriatric diseases according to the model, the way they are diagnosed, principles of prevention and treatment involving telemedicine, among project participants (verification based on a sample of participants in the focus group)	105	520	20%

chronic diseases and a reduction in the risk of complications associated with nutritional disorders, the risk of sarcopenia, or the onset of frailty syndrome. Additionally, a pilot project focused on identified risk areas, such as geriatric syndromes, sarcopenia, and malnutrition, suggests that telemedicine can be an effective tool in the prevention and treatment of specific health issues related to the unfavorable aging process. The practical aspect also includes the possibility of personalized care tailored to the specific needs of seniors. The findings from this project can serve as inspiration for further investments and initiatives in the development of telemedical technologies aimed at improving geriatric care in the Polish healthcare sector.

CONCLUSIONS

The project for the implementation and testing of pilot telemedical solutions in geriatrics represented an innovative initiative aimed at improving care for the elderly. Initiated to address challenges related to the care of the geriatric population, the project focused on effective risk management and the implementation of remedial actions in the context of modern telemedical technologies. During the project's implementation, key risk areas were identified, such as geriatric syndromes, sarcopenia, and malnutrition, with a specific focus on them during the implementation of telemedical solutions. Risk management strategies were tailored to the specifics of elderly care, considering unique challenges associated with age and health conditions.

Actions such as telemonitoring, remote medical consultations, and self-examinations were successfully implemented, bringing tangible benefits such as reducing patient waiting times for care, limiting the risk of hospitalization, and improving the efficiency of geriatric care. The effectiveness of these actions was confirmed through the analysis of demographic data and the assessment of patient progress. In summary, this project not only played a key role in advancing the field of telemedicine in the context of elderly care but also provided valuable insights into effective risk management and remedial action strategies. Its success serves as inspiration for further initiatives aimed at improving geriatric care using modern telemedical technologies.

During the pilot project, we observed that telemedicine plays a crucial role in both identifying health problems and coordinating care for seniors. Regular monitoring of health parameters allowed for early detection of potential risks and prompt action,

resulting in a 30% reduction in emergency hospital visits. Integrated telemedicine systems facilitated the coordination of care between various specialists, improving treatment outcomes and patient satisfaction. Specifically, 96% of participants expressed satisfaction with the telemedicine services, highlighting the system's effectiveness in enhancing healthcare delivery and improving the overall quality of life for seniors.

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CORRESPONDING AUTHOR

Tomasz Mazurek
Clinical Research Support Centre,
4th Military Clinical Hospital in Wroclaw, Poland
email: tmazurek@4wsk.pl

ORCID AND CONTRIBUTIONSHIP

Beata Jankowska-Polańska: 0000-0003-1120-3535 A B D F
Bartosz Sapilak 0000-0002-7999-5615 A D F
Magdalena Kałuska: 0009-0001-5142-2962 A B C D F
Tomasz Mazurek: 0009-0002-6706-962X A B C D F
Jolanta Oen: 0009-0009-1515-7346 A B C D F
Artur Janusz: 0000-0002-7651-6316 E F
Wojciech Tański: 0000-0002-2198-8789 A B D E F

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SHORT COMMUNICATION

CONTENTS

Comprehensive management of pericoronitis in lower third molars: extraction, operculectomy, and coronectomy approaches

Agnieszka Rolek[1], Piotr Pławecki[2]

[1]PRIVATE DENTAL PRACTICE – AGNIESZKA ROLEK, WROCLAW, POLAND

[2]DEPARTMENT OF CRANIO-MAXILLO-FACIAL SURGERY, PROVINCIAL SPECIALIST HOSPITAL No. 5 IN SOSNOWIEC, SOSNOWIEC, POLAND

ABSTRACT

Pericoronitis is a common dental condition involving the inflammation of soft tissues around a partially erupted wisdom tooth, typically the lower third molars. It is most frequently diagnosed in young adults aged 20-29, with a higher prevalence in women. The condition often results from the accumulation of bacteria and food debris under the operculum, leading to infection and inflammation. Common symptoms include localized pain, gum swelling, trismus, and sometimes pus discharge. This study aims to discuss the types of clinical management of pericoronitis of the lower third molar as described in the literatureTooth extraction eliminates the source of inflammation, providing long-term relief but carries a higher risk of inferior alveolar nerve damage and greater postoperative discomfort compared to other methods.This minimally invasive procedure removes the gingival cap to prevent food and bacteria accumulation. It is suitable for patients with good oral hygiene and proper tooth angulation. Electrosurgery and laser methods reduce bleeding and swelling but have contraindications. Coronectomy, an alternative to extraction, avoids nerve damage by leaving the roots in place. Suitable for non-carious, pathology-free teeth, it shows lower risk of sensory disturbances and postoperative complications, though root migration may occur. Pericoronitis requires personalized treatment based on patient condition and tooth positioning. Thorough diagnostics and tailored approaches are crucial for effective management and improved outcomes.

KEY WORDS: coronectomy, operculectomy, pericoronitis

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INTRODUCTION

Pericoronitis is a common dental condition associated with inflammation of the soft tissues surrounding a partially erupted wisdom tooth, typically the lower third molars. The condition is most commonly diagnosed in young adults aged 20-29, more frequently in women than in men [1]. The condition often arises due to the accumulation of bacteria and food debris under the operculum. This creates an ideal environment for bacterial growth, leading to infection and inflammation. Common symptoms include pain at the site of the affected tooth, swelling of the gums, trismus, and sometimes pus discharge. Diagnosing pericoronitis involves a combination of clinical examination, analyzing patient history, and, when necessary, radiographic imaging such as OPG or CBCT scans which are used to evaluate the position of the partially erupted tooth and any underlying bone involvement. The condition must be treated, and several options have been found, such as tooth extraction, operculectomy, and coronectomy.

AIM

The aim of the study was to discuss the types of clinical management of pericoronitis of the lower third molar described in the literature.

REVIEW AND DISCUSSION

EXTRACTION OF THE THIRD MOLAR

Inflammation occurring in the area of a partially erupted third molar usually causes severe pain for the patient, which, even after periods of remission, repeatedly intensifies. Extracting the causative tooth certainly eliminates the cause, which in the long term leads to permanent relief of symptoms. In cases of teeth affected by caries or with periapical changes, where other methods such as coronectomy are not applicable [2], extraction of the third molar that does not predict proper alignment in the dental arch and poses a risk of recurrent inflammation under the gingival cap often becomes the method of choice. However, before deciding on extraction, the

risk of complications such as damage to the inferior alveolar nerve, which is greater in the case of extraction than with the alternative method - coronectomy [3,4], must always be considered. Studies also indicate that postoperative discomfort after extraction of the third molar was greater than in the case of coronectomy [5].

OPERCULECTOMY

A minimally invasive method of treating pericoronitis is the removal of the gingival cap using a scalpel, laser, or electrocautery, with the aim of leaving space behind the third molar that will not predispose to food residue accumulation and will be easy to clean [1]. Patients are qualified for the gingival cap removal procedure based on radiological analysis and clinical examination. Patients with good oral hygiene, the presence of a neighboring tooth, sufficient space in the arch for the erupting tooth, and vertical angulation may be qualified for this method [1,6]. Electrosurgery methods have many advantages: they eliminate the risk of bleeding, cause less swelling, and disinfect the surgical field [1] but are contraindicated in patients with a pacemaker, after radiotherapy, or those with diabetes [6,7]. A diode laser, Er:YAG laser, CO2 laser, and Nd:YAG laser can also be used for operculectomy. Laser methods reduce the risk of bacteremia, achieve hemostasis, reduce postoperative discomfort, and shorten healing time. Researchers observed good results one month after healing in patients who underwent gingival cap removal. There were no signs of inflammatory infiltration, and the color and structure of the gum were physiological.

CORONECTOMY

First introduced in 1984 by Ecuyer and Debien [2], coronectomy is considered an alternative to the extraction of the third molar located near the inferior alveolar nerve canal to avoid damage to the neurovascular bundle. The procedure is performed on non-carious teeth, without periapical pathology, in immunocompetent patients, under local anesthesia, and involves gently cutting and then separating the tooth crown from its roots. All granulation tissues are removed with a curette, the bone edge is smoothed, the bone cavity and remaining root are irrigated with saline, doxycycline (doxycycline hyclate, 50 mg capsules; Watson Laboratories, Corona, CA) is applied, and sutures are placed [2]. After the procedure, the patient takes antibiotics for 7 days, usually penicillin VK, 500 mg orally 4 times a day or clindamycin 300 mg orally 3 times a day if allergic to penicillin, and is required to maintain special oral hygiene including tooth brushing and chlorhexidine 0.12% rinses 3 times a day [2]. This technique is dedicated to vertically, mesioangular, or distoangular impacted teeth, where the risk of nerve damage using a drill is lower than with horizontally impacted teeth [2, 3]. Studies indicate that teeth subjected to coronectomy should not undergo endodontic treatment, as this carries a much higher risk of infection of the remaining fragment [8]. In patients with a high risk of damage to the inferior alveolar nerve, there was no or incidental cases of sensory disturbances after coronectomy [3, 4]. In the case of extraction of the lower third molar with significant proximity to the inferior alveolar nerve canal, the percentage of sensory disturbances was significantly higher [4]. After coronectomy of lower third molars, researchers observed root migration towards the crown, which was fastest in the first three postoperative months and ceased between the 12th and 24th months after the procedure [2, 4].

CONCLUSIONS

Pericoronitis is a common issue among young people, often causing severe pain and reducing quality of life. Depending on individual patient conditions, general burdens, the amount of space in the dental arch, and the position of the tooth relative to anatomical structures, the method of treating pericoronitis is chosen. Both the extraction of the causative tooth and operculectomy or coronectomy yield satisfactory results and significant improvement in general and local conditions. A key aspect is thorough diagnostics and selection of the method tailored to the individual needs of the patient.

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ORCID AND CONTRIBUTIONSHIP

Agnieszka Rolek: 0009-0007-2880-2055 A B D E F
Piotr Pławecki: 0000-0003-2550-8452 A B D E F

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Agnieszka Rolek
Private Dental Practice - Agnieszka Rolek Wroclaw, Poland
e-mail: aga.rolek@gmail.com

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

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