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ORIGINAL ARTICLE

# THE IMPACT OF THE DIABETES ON ORAL HEALTH – AN OBSERVATIONAL STUDY

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**ABSTRACT**
**The aim:** To evaluate the impact of diabetes mellitus on the oral health.
**Materials and methods:** This is an observational study. In the present research were taken into consideration 300 patients. The study sample consisted of 191 males, respectively (63.7%) of them and 109 females (36.3%) of the participants.
**Results:** In the current study, we analyzed the oral health of diabetic patients within the age class of 11–80 years. We divided them into seven age groups: 11-20, 21-30, 31–40, 41–50, 51–60, 61–70, and 71-80 years. Based on the results of the current study it was observed that 83.7% of patients were affected by gingivitis. The results of our study show that dental caries prevalence was 68.7 % and the prevalence of missing teeth among patients with diabetes mellitus was 78.7%. According to the ANOVA test, high blood sugar values had a direct impact on the manifestation of gingivitis and there it was a strong correlation between them P-value = .000. Males with diabetes mellitus were more affected by dental caries and this was indicated by the P-value= .02, in comparison to females who recorded a P-value = .03. The relation between gender males and missing teeth was highly significant P-value = .001.
**Conclusions:** The present study proved that diabetes mellitus is risk factor for oral health. The authors studied this group of patients exposed to high blood glucose levels and found out that oral diseases were high in these patients.

**KEY WORDS:** age range, blood glucose values, dental caries, gingivitis, missing teeth

## INTRODUCTION

Diabetes mellitus is a metabolic disorder and its elevated global prevalence makes it the most problematic disease in the world [1].

According to the data conducted by Cho et al. the global prevalence of diabetes mellitus in 2017 was approximately 425 million (8.8%) and it is assumed to increase to 629 million by 2045 [2]. A scientific study showed that diabetes mellitus is a chronic disease accompanied by hyperglycemia and brings on to different complications, which includes the oral cavity [3].

More recently, study conducted by Nazir et al. found out that the oral complications associated with diabetes mellitus include the pathologies such as xerostomia, gingivitis, periodontal disease, missing teeth, dental caries, periapical lesions, taste and salivary gland dysfunction [4].

Daković D et al. in their study demonstrated that gingivitis and periodontitis among juveniles are chronic inflammatory diseases that have effect on the supporting tissues of the teeth in the type 1 diabetes mellitus patients [5].

In a recent study, conducted by Latti et al. is affirmed that with the increasing of the age, blood sugar levels, DMFT values and dental caries escalate in diabetics patients [6].

In a preliminary study, conducted by Wiener et al. proved that diabetes mellitus among adults was associated with having 6 or more teeth extracted [7]. Other clinicians reached at a conclusion that juveniles with T1DM have poor oral hygiene and are potentially at high risk of future oral diseases [8].

According the findings of recent study conducted by Rohani, it was showed that oral problems in diabetic patients are considered as utmost complications affecting on patients' quality of life [9]. According to Bissong et al. in their important research is stated that proper management of blood sugar levels might meliorate the oral health of patients who suffer from diabetes mellitus [10].

In a prior study conducted by Chen et al. it is declared that the persistent efforts to raise the diabetes awareness and educational programs need to take steps forward concerning juveniles in particular males [11]. In the study investigated by Newman et al. it was confirmed that type 2 diabetes mellitus is a significant risk factor for cardiovascular illness [12].

Another research conducted in the United States of America reported a 40% increment in the risk of type 2 diabetes mellitus among smokers [13].

In order to reach a significant conclusion in the present study were evaluated potential risk factors such as age, blood sugar values, number of cigarettes smoked per day and tooth brushing in diabetic patients in correlation with oral pathologies such as gingivitis, dental caries and missing teeth.

## THE AIM
The purpose of this study is to analyze the impact of diabetes mellitus on the oral health.

## MATERIALS AND METHODS
The current research was conducted in the period from February 2019 to November 2020 at the Department of Pathology in the Regional Hospital of Vlore, Albania. In the present research were taken into consideration 300 patients with an age range from 11 to 80 years old. The study sample was composed by 191 males, respectively 63.7% of them and 109 females or 36.3% of the participants.

Data collection was done using clinical documentations as well as questionnaires which were completed by the hospital nurses. There is a specialized documentation in which are registered all patients with diabetes mellitus who have been hospitalized. This documentation provides information about patients' age, gender and blood sugar values. The questionnaire included the characteristics of the sample such as gingivitis, dental caries and missing teeth.

The questionnaire was designed based on the resolution of the Albanian National Committee no. 9, date 11.11.2011. The duration of the questionnaire lasted 20 minutes and the anonymity of the participants was preserved. The original research was conducted according to the guidelines of the Helsinki statement [14]. Based on the Helsinki Declaration approved by the World Medical Association the current research, in which participants were diabetics patients, was obviously formulated based on protocols. All data were anonymous and the confidentiality of patients' medical records was respected. The participants had the right to withdraw at any time. There was no withdrawal of the patients from the research.

Inclusion criteria in the current study were the age of patients, who should be over 11 years old and all the patients who suffered from diabetes mellitus. The exclusion criteria of the study were pregnant women and non-diabetic patients who were not observed.

For each patient the following parameters were recorded such as dental caries, missing teeth and the presence of gingivitis. Depending on the gender of the patients and their age, the present study is an attempt to demonstrate the impact of diabetes on oral health, assessing the association of diabetes mellitus with gingivitis, dental caries and missing teeth.

All patients in this study suffer from diabetes mellitus and we chose this group of patients because they were the most difficult people in adhering to dietary therapy. The diabetic patients had tendency to replace energy expenditure with foods high in fats and carbohydrates. They accepted that even though had diabetes mellitus, they again consumed meat, bread, pasta, fried foods, candy, chocolate and biscuits. The patients stated that restricting these food products encourages them to eat more of those foods from which they are deprived. Furthermore, for these patients abstain from smoking resulted to be enormously difficult.

Most of these patients don't assess the risk and serious consequences of diabetes mellitus even when have repercussion for their relatives. The patients in this study except diabetes mellitus had also concomitant cardiovascular disease. It should be noted that Albania lack of medical knowledge culture and tradition.

Drug therapy in patients was 500 mg metfonorm hydrochloride 2 times daily, novonorm 2mg tablet 2 times daily, torvast 10 mg once a day, hyzaar 50-12.5 once daily, lobivon tablet 5 milligrams once a day.

## STUDY DESIGN
This is an observational study. Descriptive analysis was performed using percentages for qualitative variables such as gender, age, blood sugar values, cardiovascular disease, number of cigarettes smoked per day, tooth brushing, gingivitis, dental caries and missing teeth. The current study was approved by the University of Vlore, Albania.

## ETHICAL APPROVAL AND PERMISSIONS
Permission to collect data from the patients was obtained in the Department of Pathology at the Regional Hospital of Vlore, Albania.

## STATISTICAL ANALYSIS
Statistical analysis was performed using IBM SPSS 23.0 statistics, Microsoft Windows Linux, Chicago, IL, USA. Data were analyzed by Post Hoc LSD test in variance analysis (ANOVA). $P \leq 0.05$ values were considered significant.

## RESULTS
The sample is composed by 300 participants of which 191(63.7%) are males and 109 (36.3%) are females. The majority of patients participating in the study belonged to the age range from 41 to 50 years old, precisely 62 (20.7%) of them, while 81 (27%) of the patients were from 51 to 60 years old.

Based on the present research it resulted that 24.4% of the participants had high blood sugar values 250-300 mg/dl, whereas 19 % of the patients had very high blood sugar values 300-350 mg/dl. The results of our study showed that the most affected by cardiovascular disease were male with 188 patients or 62.7 % (Table I).

In the current study, the significant finding is that the percentage of the patients with diabetes mellitus who reported smoking 6 to 10 cigarettes per day was 48.3 % of the participants. In the present survey 52.4% of the

**Table I.** Shows the data of the participants' with diabetes and cardiovascular disease

| Variables | Age group (years old) | | | | | | |
|---|---|---|---|---|---|---|---|
| Age group | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 |
| Male | 10(3.3%) | 18(6%) | 11(3.7%) | 40(13.3%) | 56(18.7%) | 36(12%) | 20(6.7%) |
| Female | 8(2.7%) | 10(3.3%) | 12(4%) | 22(7.3%) | 25(8.3%) | 21(7%) | 11(3.7%) |
| Blood glucose values | | | | | | | |
| 150-200 mg/dL | 8(2.7%) | 7(2.4%) | 7(2.4%) | 37(12.4%) | 7(2.4%) | 17(5.7%) | 20(6.7%) |
| 200-250 mg/dL | 8(2.7%) | 3(1%) | 3(1%) | 20(6.7%) | 2(0.7%) | 13(4.4%) | 14(4.7%) |
| 250-300 mg/dL | 1(0.3%) | 4(1.3%) | 3(1%) | 16(5.4%) | 4(1.4%) | 22(7.3%) | 23(7.7%) |
| 300-350 mg/dL | 1(0.3%) | 3(1%) | 2(0.7%) | 8(2.7%) | 3(1%) | 19(6.4%) | 21(7%) |
| Cardiovascular disease | | | | | | | |
| Yes | 14(4.7%) | 16(5.4%) | 12(4%) | 37(12.3%) | 51(17%) | 31(10.4%) | 20(6.7%) |
| No | 5(1.6%) | 9(3%) | 14(4.7%) | 21(7%) | 21(7%) | 28(9.3%) | 21(7%) |

**Table II.** Shows the frequency of cigarettes smoked per day, tooth brushing and oral health complications

| Variables | Age group (years old) | | | | | | |
|---|---|---|---|---|---|---|---|
| Age group | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 |
| No. of cigarettes smoked per day | | | | | | | |
| Never smoked | 10(3.3%) | 7(2.4%) | 5(1.7%) | 6(2%) | 6(2%) | 7(2.4%) | 4(1.3%) |
| 1-5 cigarettes per day | 6(2%) | 3(1%) | 14(4.7%) | 16(5.4%) | 18(6%) | 25(8.4%) | 28(9.3%) |
| 6-10 cigarettes per day | 8(2.7%) | 7(2.4%) | 17(5.7%) | 24(8%) | 26(8.6%) | 34(11.3%) | 29(9.6%) |
| Tooth brushing | | | | | | | |
| Once a day | 13(4.4%) | 11(3.7%) | 20(6.7%) | 21(7%) | 22(7.3%) | 28(9.3%) | 42(14%) |
| 2 times a day | 8(2.7%) | 7(2.4%) | 13(4.4%) | 14(4.7%) | 14(4.7%) | 24(8%) | 25(8.4%) |
| 3 times a day | 8(2.7%) | 2(0.7%) | 5(1.7%) | 6(2%) | 6(2%) | 7(2.4%) | 4(1.4%) |
| Gingivitis | | | | | | | |
| Yes | 2(0.7%) | 1(0.3%) | 22(7.4%) | 38(12.7%) | 48(16%) | 56(18.7%) | 70(23.4%) |
| No | 16(5.4%) | 1(0.3%) | 10(3.3%) | 8(2.7%) | 9(3%) | 11(3.7%) | 8(2.7%) |
| Dental caries | | | | | | | |
| Yes | 5(1.7%) | 7(2.4%) | 46(15.4%) | 48(16%) | 44(14.7%) | 31(10.4%) | 25(8.4%) |
| No | 25(8.4%) | 13(4.4%) | 14(4.7%) | 17(5.7%) | 11(3.7%) | 9(3%) | 5(1.7%) |
| Missing teeth | | | | | | | |
| Yes | 12(4%) | 4(1.3%) | 33(11%) | 45(15%) | 54(18%) | 55(18.4%) | 33(11%) |
| No | 2(0.7%) | 5(1.7%) | 7(2.4%) | 10(3.3%) | 13(4.4%) | 15(5%) | 6(2%) |

participants declared, that they brushed their teeth only once a day. Based on the results of the current study it was observed that 83.7% of patients were affected by gingivitis.

The results of our study show that dental caries prevalence was 68.7 % and the prevalence of missing teeth among patients with diabetes mellitus in Albania was 78.7% (Table II).

In the current study, the significant finding is that 22% of patients had more than 7 teeth with dental caries and 21% of them had 5-7 teeth with dental caries, whereas 13.7% of the patients had 4-5 dental caries and the remaining 12% of them had 1-3 dental caries.

The present study showed that most of the patients 27% of them had lost more than 7 teeth and 22% of the participants had lost 5-7 teeth, whereas 17.7% of the patients had lost 4-5 teeth and only 12 % of them had lost 1-3 teeth (Table III).

This study proved that high blood sugar values had a direct impact on the manifestation of gingivitis and there it was a strong correlation between them with P-value = .000.

Gender influenced gingivitis manifestation in which males were significantly affected more by gingivitis in comparison to females. The data analysis proved that there was a stronger correlation in males in comparison to females with P- value = .000.

Higher values of dental caries in 68.7% of the patients were also influenced by high blood sugar values. Males with diabetes mellitus were more affected by dental caries and this was indicated by the P-value= .02, in comparison to females who recorded a P-value= .03.

**Table III.** Shows dental caries and missing teeth related blood glucose values

| Blood glucose values | 150-200 mg/dL | 200-250 mg/dL | 250-300 mg/dL | 300-350 mg/dL |
|---|---|---|---|---|
| | Number of teeth with dental caries | | | |
| Age group | 1-3 | 4-5 | 5-7 | >7 |
| 11-20 | 1(0.3%) | 1(0.3%) | 1(0.3%) | 2(0.6%) |
| 21-30 | 1(0.3%) | 1(0.3%) | 3(1%) | 2(0.6%) |
| 31-40 | 6(2%) | 10(3.4%) | 14(4.7%) | 16(5.4%) |
| 41-50 | 8(2.7%) | 9(3%) | 17(5.7%) | 14(4.7%) |
| 51-60 | 12(4%) | 9(3%) | 11(3.7%) | 12(4%) |
| 61-70 | 5(1.7%) | 6(2%) | 9(3%) | 11(3.7%) |
| 71-80 | 3(1%) | 5(1.7%) | 8(2.7%) | 9(3%) |
| Blood glucose values | 150-200 mg/dL | 200-250 mg/dL | 250-300 mg/dL | 300-350 mg/dL |
| | Missing teeth | | | |
| Age group | 1-3 | 4-5 | 5-7 | >7 |
| 11-20 | 2(0.6%) | 3(1%) | 3(1%) | 4(1.4%) |
| 21-30 | 1(0.3%) | 1(0.3%) | 1(0.3%) | 1(0.3%) |
| 31-40 | 4(1.4%) | 7(2.4%) | 10(3.4%) | 12(4%) |
| 41-50 | 6(2%) | 11(3.7%) | 13(4.4%) | 15(5%) |
| 51-60 | 10(3.4%) | 13(4.4%) | 14(4.7%) | 17(5.7%) |
| 61-70 | 10(3.4%) | 12(4%) | 15(5%) | 18(6%) |
| 71-80 | 3(1%) | 6(2%) | 10(3.4%) | 14(4.7%) |

**Table IV.** Correlation between blood glucose values with gingivitis, dental caries and missing teeth

| Variables | Blood glucose values | | Gender | | |
|---|---|---|---|---|---|
| | Confidence intervals ( 95%) and P-values | | | Female | Male |
| Diseases | Confidence level 95% | P-value | | P-value | |
| Gingivitis | .08 | .14 | .000 | .003 | .000 |
| Dental caries | .27 | .34 | .000 | .03 | .02 |
| Missing teeth | .31 | .38 | .000 | .004 | .001 |

The impact of high blood sugar values affects missing teeth and the correlation between them was statistically significant with P- value = .000. The relation between gender males and missing teeth was highly significant with the P -value = .001 (Table IV).

## DISCUSSION

The goal of this observational study was to report what oral diseases patients with diabetes mellitus have and to highlight whether they have high presence of diseases such as dental caries, gingivitis and missing teeth.

According the data of recent survey conducted by Henning, it was demonstrated that the worldwide prevalence of diabetes mellitus was 8.5% in 2014 [15].

In a preliminary research, it was observed that the prevalence of oral pathologies is higher in patients with Diabetes Mellitus and that is the reason why it should be monitored since in its earlier stages [16].

Based on data collected by the Institute of Public Health in Albania, experts claim that diabetes mellitus affects 7-10% of

the Albanian population, while the study conducted in 2018 by Schmidt showed that in the United States it is evaluated that 86.1 million adults have prediabetes, in addition to those who are diagnosed with diabetes mellitus [17].

Today in Albania, most people who are diagnosed with diabetes mellitus do not have a family history of diabetes. In recent decades, public health institutions have raised awareness about diabetes and screening campaigns are being held throughout Albania.

Most patients belonged to the age group 65+, but this age range changed from 51 to 65+. This is why it is important for the population to have routine checkups and understand that measuring blood sugar levels may remove the suspicion of whether or not they have diabetes.

The results of an experimental study carried out by Akcalı et al. demonstrated that diabetes mellitus was associated with the disease of gingivitis [18]. Similar data were found in the present study, 83.7% of the participants with diabetes mellitus were affected by gingivitis.

In the present survey, gingivitis in patients with diabetes mellitus is significantly higher in patients aged from 71 to

80 years, respectively 23.4% of them in comparison with patients aged from 61 to 70 years, respectively 18.7% of participants. Thus, this study shows that gingivitis disease increases with age.

The number of cigarettes smoked per day and tooth brushing were factors associated with increased gingivitis prevalence. In the present study 52.4% of the patients reported that brushes their teeth only once a day. On the basis of our data, 48.3 % of the participants smoking 6 to 10 cigarettes per day, while 36.8% of them smoked 1-5 cigarettes per day.

The large impact of diabetes mellitus as a risk factor for oral diseases is evident in both genders, but in the current study it was demonstrated that diabetes mellitus has a greater impact on the manifestation of gingivitis in men with a P-value = .000, in comparison to women with the P-value= .003.

The current study aims to highlight that 5.4% of diabetic children aged 11-20 suffer from gingivitis raising the alarm that children are at high risk of developing serious diseases in the oral cavity and this will be associated with a large number of missing teeth. The results achieved in the current study point out that type 1 diabetes affects the oral health of children. Moreover, in a scientific study conducted by Xavier et al., it was reported that 21% of the Brazilian children with DM1 and a mean age of 13 ± 3.5 years suffered from gingivitis [19].

An epidemiological study conducted in Albania by Canga et al. showed that the prevalence of dental caries was 46.62% [20]. Similarly, the study conducted by Laganà et al. revealed that 47.19% of the participants had dental caries [21].

We consider that the strong point of this study is that gives us data on a significant number of diabetes mellitus patients who has dental caries, 68.7% of them.

On the basis of our data, blood sugar values have a significant influence on the manifestation of dental caries with the P-value = .000.

Our results are supported by the study carried out from Siudikiene et al. that clearly indicated that exists a significant correlation between dental caries and high blood sugar levels [22].

Again, similar results to the present study, which report an increase in the incidence of dental caries among patients with diabetes mellitus, are also reinforced by Akpata et al., and Singh et al., in their studies [23,24].

A study conducted in Albania by Dhamo et al. showed that 15.2% of the patients had at least one missing permanent tooth [25]. The situation is worse, if we talk about people with diabetes mellitus. The current study confirmed that 78.7% of the participants had missing teeth. Based on the results of in this study, it was found out that men who have diabetes mellitus are more likely to be affected by missing teeth with P-value = .001 in comparison to diabetic women with P-value = .004.

According to Delgado-Pérez et al., diabetes mellitus is associated with higher experience of missing teeth in an open adult population in Mexico [26]. Furthermore, another survey conducted by López-Gómez et al., reached to

the conclusion that the mean number of missing teeth is higher in people with diabetes mellitus [27]. Dar-Odeh et al. claimed that missing teeth were significantly associated with diabetes mellitus with the P-value = .009 [28].

Diabetes mellitus is a serious risk factor for the development of cardiovascular diseases [29]. The data of the current research confirmed that 62.7% of the diabetic patients had cardiovascular disease.

In the present survey it was found out that there is a strong correlation between Diabetes Mellitus and oral health. We aimed to investigate effects of the impact of diabetes mellitus on oral health based on the fact that the diabetes mellitus is one of the most serious health problems in the world. The current study proved that Diabetes Mellitus increases the risk of being affected by diseases such as dental caries, gingivitis and missing teeth.

As proved by Leite et al. glycemia adversely effects on oral health, which can be devastating for the patient [30].

On the basis of the fact that blood sugar levels leads to long-term complications in the diabetic patients this research recommends the application of low-carbohydrate diets so as not to favor the growth of cariogenic bacteria and the development of carious lesions in diabetic patients.

Regarding the results of the present study it can be stated that in order to reduce acute or chronic complications from diabetes it is important that the prevention and management of diabetes must be public health priority.

Finally, we think that to give dentists the opportunity to suggest the patients to check their blood sugar levels when they have oral diseases. Consequently, the oral cavity is an excellent indicator and rapid detector of the dentists to identify other diseases in the human body.

There are some limitations that should be declared in this study, first was the small sample size that was taken under consideration. Another limitation of this study has to do with the fact that this research was limited only in the people with diabetes mellitus.

Also, the age range can be seen as a limitation because patients older than 80 years were excluded from the study.

## CONCLUSIONS

The present study proved that diabetes mellitus is risk factor for oral health. The authors studied this group of patients exposed to high blood glucose levels and found out that oral diseases were high in these patients. Gingivitis was the most common disease which affected 83.7% of the participants, followed by 78.7% of them who had missing teeth and 68.7% of the patients who had dental caries.

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ORIGINAL ARTICLE

# BIOMARKER AND ECHOCARDIOGRAPHIC CHARACTERISTICS OF HEART FAILURE IN PATIENTS HAVING ACUTE MYOCARDIAL INFARCTION COMBINED WITH DIABETES MELLITUS OF TYPE 2

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**ABSTRACT**

**The aim:** To investigate the level of B-type natriuretic peptide (BNP) and to establish its relationship with structural and functional indicators of the myocardium in patients having acute myocardial infarction (AMI), which is complicated by heart failure (HF) with concomitant type 2 diabetes mellitus (DM2).

**Materials and methods:** The study included 120 patients who were grouped by clinical diagnosis. Every patient underwent transthoracic echocardiography of the heart: left ventricular (LV) ejection fraction (EF), left ventricular myocardial mass index (LVMI), LV relative wall thickness (LVWT), BNP, HbA1c.

**Results:** LV EF was statistically significantly lower in group 2 compared with group 1. A significant difference was found. Significant difference between LVWT within indicators of groups 1 and 2 was found. There was a statistically significant increase of the LVMI in group 2 compared to group 1. Against the background of AMI, the formation of eccentric LVH prevailed in 61% cases. There was a statistically significant increase in BNP within the group of patients suffering of AMI with HF and concomitant DM2.

**Conclusions:** There was found a statistically significant increase in BNP in patients suffering of AMI with HF and concomitant DM2, which indicates a significant degree of damage to cardiomyocytes and causes an aggravating course of HF. The relationship between BNP and LV EF was revealed , which can be used to prognostic the severity of HF in this category of patients. A strong correlation between BNP and HbA1 was discovered, which indicates a burdensome unity of metabolic disorders that accelerate the development and progression of HF.

**KEY WORDS:** Myocardial infarction, diabetes mellitus, coronary heart disease, B-type natriuretic peptide, left ventricular remodeling

## INTRODUCTION

Acute myocardial infarction (AMI) is one of the leading causes of death worldwide [1,2]. European countries and the United States show examples of successful combating this problem, over 20 years mortality has decreased by more than 50% [3,2]. In Ukraine, the prevalence and incidence of coronary heart disease (CHD) is growing annually and is among the adult population – 34,9% and 26,8%, among people of working age – 26,7% and 23,1%, respectively [4]. The course and consequences of AMI determine comorbidities, including type 2 diabetes mellitus (DM2), one of the most common comorbid conditions in patients suffering of AMI. According to international registries published in the period since 2003 till 2018, the share of patients suffering of type 2 diabetes was (20-24)% of all hospitalized because of AMI, and among those cases of impaired carbohydrate metabolism – more than 50% [5,6].

Heart failure (HF) is considered to be one of the leading causes of premature death in patients having AMI. DM2 is one of the predictors of HF [7,8]. This is especially true of HF with preserved ejection fraction (EF) of the left ventricle (LV), the criterion for determining HF with preserved EF, according to the European Society of Cardiology, ranged from 40 to 50% [7,9,10]. The prevalence of HF

shows a steady upward trend around the world, especially in patients with concomitant DM2, despite the progress in the prevention and treatment of AMI [8]. Modern clinical guidelines of various associations are improving the methods of early diagnosis, prevention and individual treatment of HF [7,11].

Biological markers that reflect different pathophysiological stages of HF remain important as a powerful tool for diagnosing acute and chronic HF, stratification of patients at high risk of HF and progression, and as a likely predictor of the effectiveness of HF treatment [12]. This is especially true for patients suffering of AMI with concomitant DM2, which requires a deeper understanding of the mutually aggravating mechanisms of comorbid pathology, which lead to the development and progression of HF.

The b-type natriuretic peptide (BNP) is actively released by cardiomyocytes in response to biomechanical stretching, volume overload, myocardial damage, ischemia, necrosis, reperfusion, metabolic or toxic damage [12,13]. Studies have shown a significant role of BNP in the early diagnosis of HF. Despite the fact that this peptide is called "brain natriuretic peptide", the center of the beginning of the synthesis of BNP is the ventricular myocardium, so it is recognized as an indicator of hemodynamic stress [14].

Asymptomatic LV dysfunction is of great importance, especially for patients having metabolic disorders, which requires improved methods of early diagnosis and prevention of HF.

An additional risk of AMI in patients suffering of DM2 is hyperglycemia, which adversely affects the prognosis [8]. It is known that in the process of carbohydrate metabolism, glucose is non-enzymatically bound to proteins, including hemoglobin. Excessive non-enzymatic glycosylation, which is characteristic of hyperglycemia, alters the natural function of glycosylated proteins. Their constant excess leads to structural changes in cells and various complications inherent in diabetes. Glycosylation is mainly subject to hemoglobin A1 (HbA1), the determination of which by the method of cation exchange chromatography reveals several options: HbA1a, HbA1b and HbA1c. The most common of these is HbA1c, which accounts for approximately 60-80% of the total amount of glycosylated hemoglobin. [5]. An increase in its content in the blood leads to tissue hypoxia and the development of angiopathies, which is associated with insufficient oxygen saturation of the basement membranes of blood vessels. Determination of HbA1c is a measure of the risk of complications of diabetes and an effective means of monitoring its treatment [5].

Despite significant advances in the treatment of AMI, the mortality rate caused by HF in patients suffering of AMI in combination with type 2 diabetes remains high, with almost half of patients dying within five years of being diagnosed with HF [15]. The results of studies indicate that the prognosis in patients with HF with preserved EF and HF with reduced EF is comparable [11]. There is a constant increase in the number of such patients, which allows to identify the problem of HF with preserved LV EF and its intermediate level, as one of the non-infectious epidemics of the XXI century [15], this necessitates a deeper understanding of structural, functional and pathophysiological mechanisms of diseases. and progression of HF, which allows to optimize the diagnosis and develop a therapeutic strategy for the treatment of HF [8].

## THE AIM

1. To investigate and evaluate the level of BNP concentration in the serum of patients with AMI, which is complicated by HF in combination with type 2 diabetes and without concomitant type 2 diabetes.
2. To establish a relationship between the levels of BNP and LV EF in patients suffering of AMI, which is complicated by HF in combination with type 2 diabetes and without concomitant type 2 diabetes.
3. To study the structural and functional parameters of the heart and to establish the type of geometry in patients with AMI with concomitant diabetes mellitus2, which is complicated by heart failure and without concomitant type 2 diabetes mellitus.
4. To determine the relationship between BNP and HbA1c in patients with AMI, which is complicated by HF in combination with type 2 diabetes.

## MATERIALS AND METHODS

120 persons were examined , both males and females. The median age is 65 (59; 74) years. The diagnosis of AMI was established based on the results of clinical, electrocardiographic, biochemical indicators, according to the order of the Ministry of Health of Ukraine № 455 from 02.07.2014 "Unified clinical protocol of emergency, primary, secondary (specialized) and tertiary (highly specialized) care " [4]. According to the consensus of the American Association of Endocrinologists (AAE) and the American Diabetes Association (ADA). Hyperglycemia was considered to be an increase in venous glucose above 7,8 mmol/L. 38% (n=34) had a history of type 2 diabetes, in 62% (n=56) had no carbohydrate metabolism disorders at the time of hospitalization for AMI,  in 29% (n=26) had been diagnosed and type 2 diabetes was detected for the first time. At the first stage, all patients were divided into 2 groups according to the clinical diagnosis: group 1 – AMI complicated by HF (n=30), group 2 – patients with AMI complicated by HF and concomitant type 2 diabetes mellitus (n=60). The class of acute CH was determined by the Killip-Kimbal classification [16]. In group 1 (n=30): Killip I was diagnosed in 73% (n=22) of patients, Killip II in 20% (n=6), Killip III in 7% (n=2). In group 2 (n=60): Killip I was diagnosed in 20% (n=12), Killip II in 48% (n=29), Killip III in 32% (n=19). For comparison,  the 3-d control group (n=30) was formed of  practically healthy people who did not have type 2 diabetes and were representative according to  age and sex. All patients with AMI received therapy according to the order of the Ministry of Health of Ukraine № 455 from 02.07.2014 "Unified clinical protocol of emergency, primary, secondary (specialized) and tertiary (highly specialized) care for patients with acute coronary syndrome with ST-segment elevation", which included narcotic analgesics oxygen therapy on demand, nitrates, low molecular weight heparins  (enoxiparin), disaggregants (aspirin, clopidogrel or ticagrelor), beta-blockers (in the absence of contraindications), angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, statins. At the prehospital stage, all patients received loading doses of ticagrelor 180 mg or clopidogrel 300 mg, aspirin 150 mg, enoxoparin, depending on body weight. Patients who received systemic thrombolytic therapy were not included in the study. 87% (n=78) of patients underwent urgent coronary angiography with stenting of the infarct-dependent vessel, in 13% (n=12) coronary angiography was not performed due to patient refusal, or the presence of contraindications. Patients in groups 1 and 2 underwent a general clinical examination, general blood test, general urine test, coagulogram, creatinine and urea, total protein, total bilirubin, AST, ALT, total cholesterol, low and high density beta-lipoproteins, blood triglycerides, glucos, troponin I and MB CPK, BNP, HbA1c, transthoracic echocardiography of the heart with the determination of EF LV (Simson)%. LV myocardial mass index (LVMI) was calculated by the formula LVMI = LVM/BSA, where BSA is body surface area, LVM is LV myocardial mass,
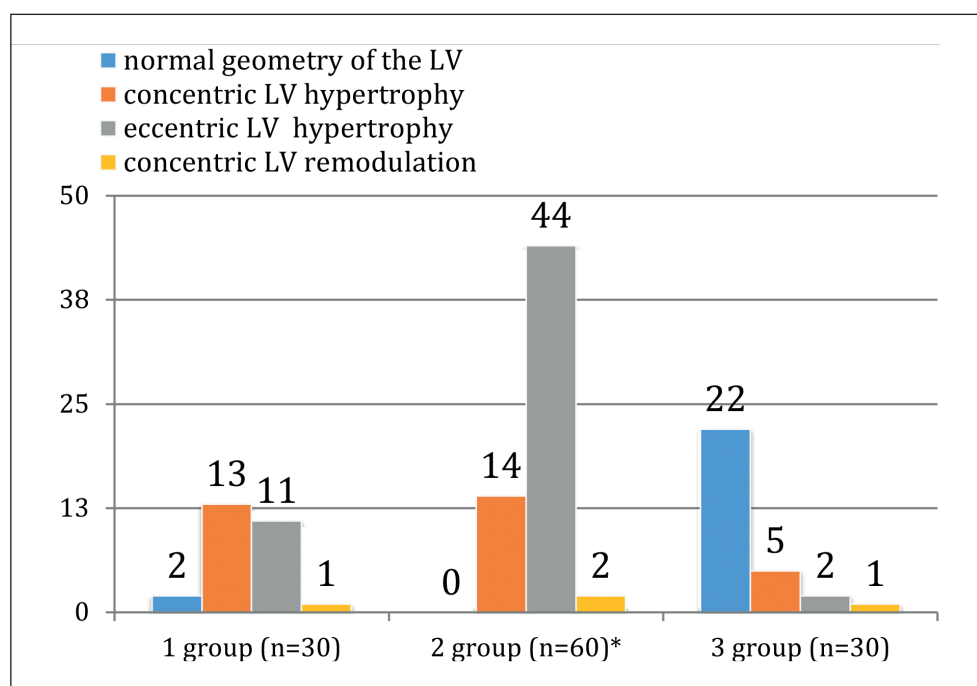
**Fig. 1.** Types of geometry of the left ventricle of the heart in the examined patients.
Note: comparison of the AMI + DM2 group with the AMI group;
* – p <0,05;

for the calculation of LVM used the formula: LVM = 1,05 [(IVST + LVPWT + EDD) 3 – EDD 3] – 14, where IVST is the thickness of the interventricular membrane in diastole, LVPWT is the thickness of the posterior wall of the LV in diastolol, EDD is the end-diastolic size of the LV. Body surface area (BSA) – according to the formula of RD Mosteller [17]: BSA (m2) = √ (weight (kg) * height (cm) ÷ 3600). To determine the type of LV remodeling, the relative wall thickness of the LV (LVWT) was determined by the formula: LVWT = (LVPWT + IVST) /EDD, where LVPWT is the thickness of the posterior LV wall in diastole; IVST – the thickness of the interventricular septum; EDD is the final diastolic size of the LV.

Types of LV remodeling were determined by A. Genau [18]:
- normal geometry of the LV (LVMI – N; LVWT <0,42);
- concentric LV remodeling (LVMI <N; LVWT> 0,42);
- concentric LV hypertrophy (LVMI > N; LVWT > 0,42);
- eccentric LV hypertrophy (LVMI > N; LVWT <0,42).
The control group underwent bicycle ergometry to exclude coronary heart disease, HbA1c, BNP, echocardiography with the determination of LV EF, LVWT, LVMI.

The study did not include patients with concomitant myocardial damage: unstable angina, secondary hypertension, persistent atrial fibrillation or flutter, dilatation, hypertrophic and secondary cardiopathy, acute left ventricular failure: Killip IV, autoimmune hematological diseases, mental disorders, endocrine disorders (except for patients with DM), patients with acute renal and hepatic insufficiency, agonists and cancer patients.

The type of distribution of the obtained data was performed according to the Shapiro-Wilk criterium. Parametric statistics methods were used when distribution was normal, in particular one-way analysis of variance (ANOVA) with Bonferroni correction. When the data had a distribution different from normal, the Kraskel-Wallis cri-

terium with the Dunn test was used. The obtained data are presented as the mean value with standard error (M±m) or median and interquartile range (Me (Q25-Q75) according to the type of distribution. The correlation was analyzed by the Spearman's test, because one of the variables had not normal distribution. Differences between groups were considered statistically significant with a probability of error of the null hypothesis p<0,05, which is common in biomedical studies.

## RESULTS

Analysis of echocardiographic data showed that the left ventricular ejection fraction (Simson),% (LV EF) was statistically significantly lower in patients of group 2 compared with group 1 and a significant difference was found (p<0,05) in patients of LV EF group 2 decreased by 10% (p<0,05) compared to patients of group 1 (Table 1). When estimating the relative wall thickness of the left ventricle (LVWT), a significant difference was found between the indicators of groups 1 and 2 (p<0,05) (Table I). The data obtained and their analysis indicate significant structural and functional disorders in the left ventricular heart muscle, which determines the severity of AMI and confirms the negative impact of type 2 diabetes on the development and prognosis of HF, so control over LV remodeling processes, both acute and the postinfarction period needs more detailed attention.

The division of patients according to the types of LV geometry of the heart is illustrated in Figure 1. In group 2 there is no normal LV geometry, which provides better diastolic filling and systolic emptying. It was found in only 6,7% of respondents in group 1. Against the background of AMI, the formation of eccentric LVH prevailed – in 61% of cases in both groups. Eccentric LV hypertrophy was

**Table I.** Indicators of trasthoracic echocardiography of the heart in patients having acute myocardial infarction complicated by heart failure with and without concomitant diabetes mellitus.

| Indicator | 1 group – AMI (n=30) | 2 groups - AMI + DM2 (n=60) | 3 groups (n = 30) |
|---|---|---|---|
| LV EF (Simson),% | 49,1±1,8 | 44,1±1,6* | 58,1±3,0 |
| LVWT | 0,44±0,01 | 0,47±0,01* | 0,36±0,03 |
| LVMI, g/m 2 | 122,2±7,2 | 134,5±8,4* | 85,1±8,5 |

Note: comparison of the AMI + DM2 group with the AMI group; * – p <0,05;

**Table II.** Glycosylated hemoglobin in patients with acute myocardial infarction complicated by heart failure with or without concomitant type 2 diabetes.

| Indicator | 1 group – AMI (n=30) | 2 groups - AMI + DM2 (n = 60) | 3 groups (n = 30) |
|---|---|---|---|
| HbA1c,% | 5,4±0,3 | 7,5±0,4* | 5,1±0,2 |

Note: comparison of the AMI + DM2 group with the AMI group; * – p <0.05.

**Table III.** Indicators of natriuretic peptide in patients with acute myocardial infarction complicated by heart failure with or without concomitant type 2 diabetes mellitus.

| Indicator | 1 group AMI (n=30) | | | 2 groups AMI + DM2 (n=60) | | | 3 groups (n=30) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Me | 25% | 75% | Me | 25% | 75% | Me | 25% | 75% |
| BNP, pg/ml | 325,3 | 302,2 | 348,4 | 423,7* | 364,6 | 482,8 | 45,4 | 37,6 | 53,2 |

Note: comparison of the AMI + DM2 group with the AMI group;* – p <0.05;

more often registered in group 2 than in group 1 by 29,5%. Most patients in group 1 had concentric LV hypertrophy. Comparison of LVMI and LVWT with BNP, which reflects the course of HF, showed a higher incidence of acute heart failure above Killip II in patients with concomitant type 2 diabetes with eccentric LV hypertrophy, which prevailed over data in concentric LV hypertrophy by 49% (p<0,05) (Figure 1).

In patients with AMI, left ventricular hypertrophy was detected in 98%. At AMI in patients with concomitant type 2 diabetes mellitus 33,3% more often registered areas of LV hypokinesia, thinning of certain segments of the posterior wall of the LV and IVST, 14% developed LV aneurysm. The obtained data indicate the severity of HF in patients with comorbid pathology and confirm the negative impact of type 2 diabetes on the structural and functional parameters of the heart.

A comparison of glycosylated hemoglobin (HbA1c) in groups 1 and 2 revealed a significant difference between the indicators (p <0,05), which indicates the presence of diabetes and confirms the negative impact of carbohydrate metabolism disorders on the structural and functional parameters of the heart (Table II).

When comparing the natriuretic peptide (BNP) of groups 1 and 2, a statistically significant increase in BNP was found in the group of patients with AMI with HF and concomitant diabetes mellitus (p<0,05) (Table III). This indicates LV myocardial ischemia, impaired myocardial structure and function and confirms the negative impact of carbohydrate disorders on the course of HF, which is confirmed by a significant deterioration in echocardiography in the group of patients with AMI with concomitant type 2 diabetes.

The comparison of parameters between both experimental groups and control group have demonstrated statistically significant differences which are caused by main disease. In second experimental group its comorbidity with DM2 have been considering as influencing factor of these findings.

The correlation analysis revealed a strong relationship between BNP and HbA1c (r=0,713, p=0,009), which indicates the unity of the process of development and course of HF in patients with AMI with concomitant type 2 diabetes.

## DISCUSSION

Manifestations of HF depend on LV remodeling, which includes damage to heart cells and extracellular matrix, followed by cell hypertrophy, apoptosis and necrosis of cardiomyocytes, activation and proliferation of fibroblasts, leading to widespread fibrosis and myocardial dysfunction. This process is characterized by progressive ventricular dilatation followed by systolic dysfunction with decreased EF. Early remodeling is characterized by stretching and thinning of the myocardium, dilatation and spherification of the left ventricle. Under these conditions, excessive stretching of the viable myocardium compensates for the decrease in myocardial mass that develops as a result of the lesion. Much attention is paid to patients having heart failure with preserved EF in which have a completely different phenotype and biochemical features different from traditional ones. Therefore, there is a need to identify their characteristics and predictors of negative myocardial remodeling in patients with AMI. This is especially true for patients with metabolic disor-

ders, namely with concomitant diabetes mellitus 2. It is proved that DM2 is a key marker of risk of developing diabetic complications, especially HF [2].

Due to the peculiarities of pathophysiological processes that lead to the development of heart failure as a result of myocardial remodeling after AMI, there is a need to stratify the risk of prognostic myocardial changes based on certain parameters. In addition to the clinical characteristics included in the prognostic scales, BNP is used as a biological marker of the clinical severity of HF.

BNP is a biomarker that has high laboratory sensitivity and specificity and can be used for early diagnosis in order to optimize the choice of treatment, monitoring the effectiveness of therapy

and predicting the consequences of the disease in patients. Its increase at the beginning of the ischemic process in the myocardium before complications depends on the degree of damage to myocardial cells, as evidenced by the deterioration of echocardiography and has a close correlation with HbA1c, which confirms the negative impact of metabolic disorders on the development of HF and evidence of unity.

The study confirms that the degree of LV dilatation in patients with AMI is closely related to comorbidities, in this case DM2. LV dilatation plays an important role in the development of HF. This study confirms a positive correlation between the level of BNP and LV EF, which allows to predict further changes in the geometry, LV function with the development and progression of HF. According to our data, the level of BNP was higher in patients with AMI and concomitant type 2 diabetes, and LV EF was much lower, which confirms the negative impact of metabolic disorders on the development and further progression of HF in this comorbid pathology.

Determination of LVMI and LVWT made it possible to distinguish between LV geometry types in the examined patients. The results of the study confirmed that heart remodeling in patients with AMI is complex and heterogeneous process that requires an individual approach. From our point of view, taking into account indexed indicators provides a better and more individual analysis.

## CONCLUSIONS

1. A statistically significant increase in the level of the biomarker of natriuretic peptide in patients with comorbid pathology (AMI with HF and concomitant diabetes mellitus), which indicates a significant degree of cardiomyocyte involvement and causes a severe course of HF has been established.
2. The relationship between BNP and LV EF, which can be used to prognostically assess the severity of HF in this category of patients has been revealed.
3. In patients with AMI, LV geometry is interrelated with the presence of comorbid pathology and confirms the negative impact of type 2 diabetes on the structural and functional parameters of the heart, which determines the severity of HF.

4. A strong correlation between natriuretic peptide and glycosylated hemoglobin in patients with comorbid pathology has been established as a basic biomarker, indicating the aggravating unity of metabolic disorders that accelerate the development and progression of HF.

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ORIGINAL ARTICLE

# PATTERN OF KRAS GENE EXPRESSION IN IRAQI WOMEN OVARIAN CARCINOMA

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## ABSTRACT
**The aim**: The goal of this study was to detect if KRAS gene and levels of had any clinical significance in the ovarian cancer by measuring levels of KRAS mRNA.

**Materials and methods:** The investigation was conducted on 84 tissue samples from newly diagnosed patients with ovarian cancer. Twenty-eight tissue sections with benign ovarian tumors were used as a control group. The qRT- PCR technique was used for measuring and analyzing levels of KRAS mRNA.

**Results:** Relative increasing of KRAS mRNA level in cancer samples was statistically significant (P<0.01) when compared to benign tumors. Statistically no significant differences were found between KRAS mRNA levels and menopausal status or family history. Gene expression has been substantially connected with age groups as the highest levels of KRAS mRNA was recorded in patients with age 50-74 years (P<0.01). Endometrium tumors exhibited significant correlations (P<0.01) across histopathological tumor types. In correlation with tumor stages, stage I was substantially linked compared to stage I (P<0.01).

**Conclusions:** It was concluded that over expression of the KRAS gene is linked to early stages of ovarian cancer, which implying that mRNA levels could be used as a diagnostic and predictive factor for ovarian cancer. More research with larger groups of ovarian cancer specimens in both primary and advanced stages is needed.

   **KEY WORDS:** Ovarian cancer, CA ovary, KRAS, KRAS mRNA, Gene Expression

## INTRODUCTION

In women, ovarian cancer represented the fifth leading cause of death causes related with cancer. It has been estimated that the survival rate is lower than fifty percent 30%-40% only [1]. The incidence ratio of epithelial ovarian tumor is one for each 72 women [2-3], with increasing rate in developing countries. Three categories of ovarian tumors have been identified: epithelial 60%, germ cell 30%, and sex-cord stromal 8%, the beginning of malignant ovarian carcinoma in vast majority of cases 80-85%, occurs in ovarian epithelium [4-5]. Absence of symptoms make it difficult to diagnose epithelial ovarian tumors during early stages, the asymptomatic condition is due to certain factors that related to clinical and histopathological features [6]. Losing of portent lesions and their evolution, is the reason why vast majority of patients with ovarian tumors are diagnosed at advanced stages with poor prognosis [7-8]. Over the years many genes/proteins have been identified as fundamental genes in carcinogenesis as they play a key role in the development and progression of various cancer types. RAS genes and their products are among the key genes that frequently mutate in human cancers which make them difficult to be targets for cancer therapy [9]. KRAS is a member of the RAS GTPase family. This gene involved in cells proliferation and apoptosis among variety of biological processes including [10]. Genetic variation in the exons of KRAS gene may associate with protein hyperactivity. Increasing activity of protein is an oncogenic mutation that very common human cancer. This sort of mutations was detected in a variety of human cancers including 17% of lung cancer, and 14% of ovarian cancer [11]. Previous studies have been providing substantial information that clarifies the role of KRAS in ovarian physiology and pathology, one of that information that the activation of RAS genes is regulated within ovarian surface epithelial cells as well as granulose cells. This activation is definitive for initiation of luteinizing hormone pathway which is involved ovulation. As a result any genetic variation that leading to mutation or over expression in the active form of KRAS gene leads to interception of granulose cell cycle and then deteriorate follicle growth [12]. On the other hand, over expression of KRAS gene in ovarian surface epithelial cells that have deficiency with Pten gene may responsible for the development of serous epithelial adenocarcinoma. Other studies reported that the over expression of mutant KRAS increase the cells susceptibility to tumorigenesis and stimulate senescence of certain ovarian cells particularly epithelial and fibroblast cells [13]. Goal of this study is to detect if the KRAS gene and its mRNA expression levels in ovarian cancer patients had any clinical significance.

## THE AIM

The goal of this study was to detect if KRAS gene and levels of had any clinical significance in the ovarian cancer by measuring levels of KRAS mRNA.

## MATERIALS AND METHODS

To implement the present study, tissue sections from eighty four patients who already diagnosed with ovarian carcinoma at different stages, have been used. Tissue biopsies are obtained from certain Iraqi hospitals after the patients undergo any of the following surgeries: Total abdominal hysterectomy with bilateral salpangio opherectomy (TAH-BSO), subtotal abdominal hysterectomy, vaginal hysterectomy, and endometrial. For a control 28 tissue sections of benign tumors were included. Tissue sections then maintained and subjected to molecular analysis. Demographic and pathological information were collected from hospitals records.

## RNA EXTRACTION, REVERSE TRANSCRIPTION

Formalin fixed paraffin-embedded tissue sections were subjected to sectioning and then RNA extraction using RN easy FFPE kit for RNA purification (Qiagen – USA). Total RNA has been reversed using the reverse transcription Thermo-Script TM (Invitrogen/United States) kit. This procedure was conducted in the 50 μl reaction volume composed of 15 μl denaturized RNA, 0.2 μl Random hexamere primers 3μg/μl, 5μl of 10 μldNTP Mix, 10μl of 5x cDNA synthesis buffer, 2.5μl RNase OUT (40U/μl), 2.5μl Thermo Script RT (15 units/μl), 14.8μl DEPC-treated water. The samples were then placed in a 96-pit thermal cycler, cycling at 25ºC for 10 minutes, 10 minutes at 37ºC, 60 minutes at 42ºC, followed by 75ºC for five minutes. The converted cDNA has been stored at -80ºC and used to amplify Kras as a PCR template.

## QUANTITATIVE REAL-TIME PCR

Real-time PCR was performed using the SYBR Green Supermix Kit (Qiagen – USA), Kras primers that described previously by Latif AH [14]., were utilized in this study, for PGK1 primers were forward:

GCGTCAAGGTGAAGATAATACCTAA,

And reverse:

CATTTAAACTTGTGGTTGCTCTT

The Applied Biosystems 7900 equipment was used to run quantitative real-time PCR tests in duplicate. Reaction volume of 20 μl was used for PCR reaction. It was contain of SYBR Green master mix (10 μl), primer mixes (1 μl), RNase free water (5 μl0, and cDNA template (4 μl). Conditions of Real-Time PCR was as follows: stage 1: 50 ºC for 2 min., stage 2: 95 ºC for 10 min., stage 3: a two-step cycle process (95 ºC for 15 sec. and 65 ºC for 1 min.) repeated for 6 cycles, and stage 4 in a two-step cycle procedure (95ºC for 15 Sec. and annealing 61ºC for 1 min.) repeated for 40 cycles. The endogenous reference gene (*PGK1*) was used as a control gene.

## DATA ANALYSIS

Results of RT-PCR analyzed using $2-\Delta\Delta Ct$ method [15]. Comparative standard curves were used to assess the efficiency (E) of qPCR amplification for target and reference genes, figure (1). Serial dilutions of reaction materials that included cDNA of reference sample and study primers were used for standard curve generation. Ct values against logarithmic values plotted for estimation proportional of cDNA copy numbers in the reference sample and stability and efficiency of primers. Slope and equations of effectiveness (E) were used as following:

E = (10 $^{-1}$/slope −1) × 100

E = (10 $^{-1}$/3.35 −1) × 100.

The cycle's threshold (Ct) is defined for each sample as the number of PCR cycles required for the fluorescence defined by the user. The Ct is inversely proportional to both the target (Kras) and reference (PGK1) genes. The level of Kras mRNA was determined by the following equations:

Logarithmic copy no. $_{(PGK1)}$ = (Ct-32.85) /-3.3592

Copy no. $_{(PGK1)}$ = 10^Log copy

Logarithmic copy no (Kras) = (Ct- 37.437) /- 4.4513

Copy no (Kras) = 10^Log copy

Fold change = copy no. ((Kras)/copy no. $_{(PGK1)}$)

## STATISTICAL ANALYSIS

The SAS (2012) program for the Statistical Analysis System was employed to determine the impact on study parameters of various variables. The smallest difference – LSD (ANOVA Variation Analysis) or T-Test – has been used in this research in order to compare the means.

## RESULTS

Eighty four samples with ovarian cancer and 24 samples with benign ovarian tumors that used as a control group were studied for the expression Kras. The median age was 47 years old and the patients were 14-70 years old. All samples of ovarian cancer have been negative for family history, according to the family's history. The clinical features of ovary cancer samples are listed in table (I). With respect to menopause, 36(46.1%) of the samples were premenopausal, while 42(53.9%) of these were postmenopausal. According to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system, the majority of samples 57(73%) were in stage I, while the other 21(27%) were at stage III. The samples have been divided into five clinical groups according to tumor histology types; Serous epithelial tumors 33(42.3%) samples, mucinous epithelial tumors 15(19.2%) samples Endometriod tumors 21(26.9%) samples, germ cell tumors 6(7.695%) samples and 3(3.84%) of Burner tumors. Table (II) shows that in both malignant and benign tumor Kras mRNA was expressed. In ovarian cancer samples the highest Kras mRNA levels were statistically important in comparison to benign tumor (mean ± SE: 661, 51 ± 75, 09, **P≤0.01), respectively (mean ± SE: 7, 82 ± 0.22). The present study shows statistically no significant differences in gene expression levels with menopause and family history, while significantly associated with age groups, since the highest levels of gene expression in patients aged between 50-74 years (mean ± SE: 1445.96 ± 82.37, **P≤0.01). In correlation with the histopathological type of ovarian tumors Endometriod tumors showed statistically significant difference in the level of Krasgene expression (Mean ± SE: 2469.55 ± 137.06, **P≤0.01)
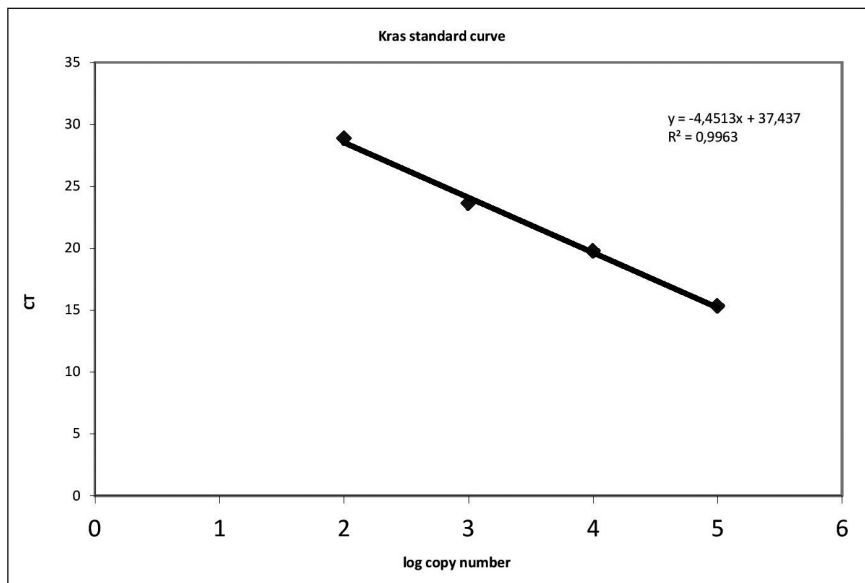
**Fig. 1.** Standard curves for qPCR efficiency calculation of target Kras gene

**Table I.** Clincopathological characteristics of patients with ovarian carcinoma

| Age groups | |
|---|---|
| 0-14 years (Children) | 9(10.7%) |
| 15-24 years (Teenagers and young adults) | 3(3.57%) |
| 25-49 years (Adults) | 33(39.28%) |
| 50-74 years (Old age) | 39 (46.42%) |
| **State of menopause** | |
| Premenopausal no. (%) | 40(47.6%) |
| Postmenopausal no. (%) | 44(52.4%) |
| **Family history** | |
| Positive no. (%) | 0 |
| Negative no. (%) | 84(100%) |
| **Tumor histological types** | |
| Serous epithelial tumors | 34(40.47%) |
| Mucinous epithelial tumors | 20(23.8%) |
| Endometriod tumors | 21(25%) |
| Germ cell tumors | 6(7.14%) |
| **FIGO stages** | |
| Stage I no. (%) | 63(75%) |
| Stage III no. (%) | 21(25%) |
| Burner tumours | 3(3,57% |

compared with other histopathological tumor type. Statistic difference between the 57(73%) stage I samples with the highest expression level (Mean ± SE: SE: 831.78 ± 71.55, **P≤0.01) and the 21(27%) stage III samples (Mean ± SE: 59.45 ± 3.16) were observed according to the tumors stage.

## DISCUSSION

A link between RAS family genetic variation like mutation or over expression and tumor lesions with advanced degree,

have been recorded by only a few studies. In the present study, relative KRAS gene expression levels were evaluated in 84ovarian tissues obtained from patients with benign and malignant tumors using the real-time PCR method. Ovarian cancer samples in comparison to benign tumor. Similarly to this result, Hong et al [16] reported that there was almost no p21Ras expression in normal breast tissue, but high level of expression in breast cancers. Latif [14] also detected that the mRNA of KRAS gene was highly expressed in samples of colorectal cancer comparing with controls. In correlation with clincopathological parameters, the present study shows that the relative levels of KRAS mRNA and older age of patients were significantly associated. Endometriod tumor histological type and primary stage of tumor (stage I). Except for the age of patients, Pzik et al. [17] found that none of the clincopathological parameters they looked at (patients' sex, age, and tumor histological type, stage, grade, and chemotherapy treatment) were significantly linked to KRAS expression and levels of KRAS mRNA. Wan et al. [18] found significant associations between KRAS expression and cancer patient age, with the highest levels of KRAS mRNA found in patients aged over 56 years, which is similar to the current study's findings. Their findings, on the other hand, contradicted the findings of the current study, as they discovered a significant link between high levels of KRAS mRNA and poor tumor differentiation. The current study's findings contradicted those of Birkeland et al. [19], who discovered that high KRAS mRNA levels were significantly associated with advanced FIGO stage, non-Endometriod histological type, high grade, and lymph node metastasis. What is interesting about the findings of the present study is that the KRAS gene over expression increased remarkably in the subgroup of patients over 50 years of age. Also what is interesting the association of KRAS over expression KRAS with early stage of ovarian cancer which is conflict with results of most previous studies that demonstrated the correlation between KRAS over expression and late stages

**Table II.** Relative expression levels of KRAS mRNA in ovarian cancer patients, compared to clincopathological features

| KRAS gene expression | |
|---|---|
| Tumor group | Mean ± SE of KRAS gene |
| Benign ovarian tumors | 7.82 ± 0.22 |
| Malignant ovarian tumors | 661.51 ± 75.09 |
| T-test | 47.911** |
| ** (P≤0.01). | |
| Age groups | Mean ± SE of KRAS gene |
| children age 0-14 years | 87.46 ± 4.07 |
| Teenagers and young adults aged 15-24 years | 5.76 ± 0.63 |
| Adults aged 25-49 years | 3.98 ±0.42 |
| Adults aged 50-74 years | 1445.96 ± 82.37 |
| LSD Value (P-value) | 52.269 **(0.0001) |
| **P≤0.01 | |
| Histological tumor type | Mean ± SE of KRAS gene |
| Mucinous | 10.48 ± 1.94 |
| Serous | 36.58 ± 2.66 |
| Endometriod | 2469.55 ± 137.06 |
| Germ cell tumor | 35.52 ± 1.83 |
| Burner tumor | 2.692 ± 0.39 |
| LSD Value (P-value) | 23.605 **(0.0001) |
| ** (P≤0.01) | |
| Tumor stage | Mean ± SE of KRAS gene |
| Stage 1 | 831.78 ±71.55 |
| Stage 3 | 59.45 ±3.16 |
| LSD Value (P-value) | 37.205 **(0.0001) |
| **P≤0.01 | |

of tumor. The majority of previous research has focused on the frequency of mutations in the KRAS gene. They claim that the KRAS gene is frequently mutated in various types of tumors and plays a role in cell proliferation, apoptosis, migration, and differentiation. KRAS expression has been studied for its prognostic value in a variety of cancers, but the majority of studies have found a link between KRAS gene over expression and advanced tumor stages. To our knowledge, the present study is one of the few studies that have found a link between KRAS gene over expression and tumor development in the early stages. In general, over expression of Ras genes has been linked to a poor prognosis for colon carcinoma, leading to the conclusion that Ras over expression cannot be used as a predictive factor [20]. On the other hand, another study on the lesions of cervix adenocarcinoma as found that Ras genes were over expressed, but no link between Ras over expression and prognosis was discovered [21].The current study concluded that over expression of the KRAS gene is associated with early stages of ovarian cancer, implying that mRNA levels of this gene could be used as a diagnostic and predictive factor

for ovarian cancer. Further studies with a larger cohort of ovarian cancer specimens in both primary and advanced stages are needed to confirm these findings.

## CONCLUSIONS

The present study concluded that over expression of the KRAS gene is linked to early stages of ovarian cancer, which implying that mRNA levels could be used as a diagnostic and predictive factor for ovarian cancer. To confirm these findings, more research with larger groups of ovarian cancer specimens in both primary and advanced stages is needed.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

# COMPARATIVE ANALYSIS OF ENDURANCE CEMENTS FOR THE FIXATION OF NON-REMOVABLE ORTHOPEDIC CONSTRUCTIONS UNDER THE ACTION OF CYCLIC COMPRESSION

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**ABSTRACT**
**The aim:** To conduct studies of the physical and mechanical properties of cements for fixation, namely the duration of cement samples under cyclic compression.
**Materials and methods:** We have conducted a study of 6 cements for permanent fixation of fixed structures of dentures. To study the duration of cements under cyclic compression, a special stand was used, which allows testing samples for cyclic compression with a load frequency of 5.4 Hz or 324 cycles per minute and an increase in compression in a cycle from 10 to 100 kg/s.
**Results:** Comparative analysis of the mechanical properties in compression of dental cements for permanent fixation showed that the obtained deformation curves differ significantly in each of the cement samples used in the study.
**Conclusions:** The results of the study of the physical and mechanical properties of cements indicate that the most optimal complex of properties is possessed by the zinc-phosphate cement Unitsem and glass ionomer cements Cemion and Fuji I. This is most clearly confirmed when studying the duration of cement samples under cyclic compression, which simulates a real situation.

   **KEY WORDS:** Dental Cements, dental crowns, fixation, cyclic compression

## INTRODUCTION
The frequency of using fixed dentures is distributed as follows: 37.7% – single crowns, 29.4% – bridges, 1.45% – pin teeth, 0.7% – inserts [1, 2]. The average term of use of fixed orthopedic structures is 5-6 years, of which bridges are 4-5 years, single crowns are 6 years [3, 4].

An important reason for the short use of fixed prostheses is the cementation of crowns, which accounts for 21% of all complications [5-7].

There are many reasons for short-term use of fixed dentures. The leading causes that lead to complications and dysfunction and cementation of fixed orthopedic structures are as follows:
   1) low crowns of abutment teeth due to improper preparation;
   2) violation of the prosthesis manufacturing technology and, as a result, loose fit of the crown edges to the tooth neck;
   3) prosthesis breakage;
   4) violation of the rules of mixing fixing cement;
   5) violation of fixation technology [8-10].
The main factors that affect the effectiveness of fixation of fixed dentures include: the correct preparation of the abutment teeth, the quality of the materials used, as well as adherence to the rules and technology of fixation [11-13].

According to the requirements, cements for fixation must be sufficiently resistant to the influence of the oral environment and provide a strong bond through mechanical adhesion and adhesion [14, 15].

A wide variety of cements are used in modern dental practice. However, there is not enough information in the literature on the effect of cement on the tissues of the oral cavity, therefore, we consider it expedient to study the biological effects of various types of cements on the tissues of the oral cavity, especially on the tissues of the tooth and the body as a whole.

## THE AIM
The aim of the research was to conduct studies of the physical and mechanical properties of cements for fixation, namely the duration of cement samples under cyclic compression.

## MATERIALS AND METHODS
This study was conducted at the orthopedic dentistry department I. Horbachevsky Ternopil National Medical University, Ukraine, and was approved by the ethics committee of the I. Horbachevsky Ternopil National Medical University.

A study was conducted on 6 cements for permanent fixation of non-removable denture structures. Of these, 2 are zinc-phosphate cements: "Adhesor Fine" ("SpofaDental", Czech Republic) and "Unitsem" ("VladMiva", Russia); 2 polycarboxylate cements "Belokor" ("VladMiva", Russia); "Adhesor Carbofine" ("SpofaDental", Czech Republic); 2 glass ionomer cements: " Cemion F" (VladMiva, Russia) and "Fuji I" (GC, Japan).6 batches of samples (3 samples per batch) were made for testing. The sample is a removed tooth with a solid metal crown fixed on it by means of the investigated cements.

To study the duration of cements under cyclic compression, a special stand was used, which allows testing samples for cyclic compression with a load frequency of 5.4 Hz or 324 cycles per minute and an increase in compression in a cycle from 10 to 100 kg/s.

For the study, we selected three levels of maximum compression forces 25±1 kg/s, 40±1 kg/s, 60±1 kg/s, which determined the cyclic duration of the samples. The duration is expressed in the number of cycles that the sample withstands until failure at the selected load levels. For the destruction of the sample, the loss of the cement-containing properties was taken, which is expressed in a slight displacement of the crown relative to the tooth when a weak static force (~ 2 – 3 kg) is applied to detach the crown from the tooth.

During the test of the sample, the load was interrupted to determine the level of destruction. For this, the sample was removed and examined under an MBS-9 microscope the state of the cement film between the edge of the crown and the tooth. After that, holding the tooth, an attempt was made to recover the crown using a metal hook and a dosed load. The tests were stopped and the number of fracture cycles was recorded at the moment when the crown was displaced relative to the tooth. Checking the integrity of

the connection of the crown with the tooth was observed under a microscope in the following mode: up to $10^4$ cycles for every thousand cycles; $2 \times 10^4$ – every two thousand; to $5 \times 10^4$ – after five thousand and further to destruction or termination of the test every ten thousand cycles.

## RESULTS

The obtained deformation curves differ significantly for different types of cements that were used in the experiment. As a result of the study, the following results were obtained.

Fracture is fragile in zinc-phosphate cements "Adhesor Fine" and glass ionomer cement "Fuji I", they do not exhibit plasticity, their deformation curves have only a linear elastic section.

The investigated polycarboxylate cements "Belokor", "Adhesor Carbofine" and glass ionomer cement "Cemion F" shows significant plasticity with a pronounced yield point, which corresponds to the beginning of deviation from the linear section.

Further, the deformation pressure increases, and when it reaches a maximum, plastic deformation can be several percent. The pressure drop during the subsequent plastic deformation is associated with the formation of stable shear bands, which arise due to the destruction of crosslinking in the corresponding slip planes.

As a result, these processes lead to an increase in the cross-section of the sample (the so-called barrel-shaped sample is observed), which leads to the need to increase the deforming forces to support plastic deformation.

"Unitsem" zinc phosphate cement occupies an intermediate position between the two types of deformation curves described above. In contrast to zinc-phosphate cement "Adhesor Fine" and glass ionomer cement "Fuji I", the curve created as a result of the experiment shows a transition from elastic deformation to the stage of plastic deformation. However, upon reaching plastic deformation within a few percent, destruction of the sample occurs (Table I).

For "Adhesor Fine", "Fuji I" and "Unitsem", this value was determined as the pressure at the time of destruction of the sample, and for "Belokor", "Adhesor Carbofine" and "Cemion F" – as the pressure at the maximum of the deformation curve. It is seen that all these values are in the range of 800-1600 kg/cm$^2$.

Analysis of the compressive strength data for all studied cements showed that for "Adhesor Fine", "Fuji I", and

**Table I.** Kinds of cements and their strength limits (M+m)

| № | Name of cements | Strength limit (M+m) |
|---|---|---|
| 1 | "Unitsem» | 1030 ± 40 |
| 2 | "Adhesor Fine» | 1590 ± 60 |
| 3 | "Belokor» | 840 ± 40 |
| 4 | "Adhesor Carbofine» | 820 ± 30 |
| 5 | "Cemion» | 1360 ± 50 |
| 6 | "Fuji I» | 1210 ± 50 |

**Table II.** Endurance indicators of cement samples under cyclic compression

| № | Name of cements | $P_{max}$ ( H ) | | |
|---|---|---|---|---|
| | | 250 | 400 | 600 |
| 1 | "Unitsem» | $(9\pm1)\times10^8$ | $(1\pm0,14)\times10^7$ | $(9\pm1)\times10^5$ |
| 2 | "Adhesor Fine» | $(4\pm0,48)\times10^5$ | $(1\pm0,14)\times10^5$ | $(3\pm0,26)\times10^4$ |
| 3 | "Belokor» | $(7\pm0,69)\times10^5$ | $(1,5\pm0,16)\times10^5$ | $(3,5\pm0,41)\times10^4$ |
| 4 | "Adhesor Carbofine» | $(12\pm1,18)\times10^5$ | $(2\pm0,38)\times10^5$ | $(4,5\pm0,51)\times10^4$ |
| 5 | "Cemion» | $(30\pm3,12)\times10^5$ | $(3,5\pm0,41)\times10^5$ | $(5\pm0,53)\times10^4$ |
| 6 | "Fuji I» | $(1\pm0,14)\times10^8$ | $(5\pm0,53)\times10^6$ | $(3,5\pm0,41)\times10^5$ |

"Unitsem", this value was determined as the stress at the moment of sample failure, and for "Belokor", "Adhesor Carbofine", and "Cemion F", as the stress at the maximum of the deformation curve.

Thus, all the studied cements have rather significant parameters of strength, but the presence of plastic deformation in some materials suggests that it is polycarboxylate cements that should have higher functional properties. Due to the fact that the load in bridges is greater than in single crowns, zinc phosphate and glass ionomer cements should be used when fixing them.

In the course of the study, we established the endurance indicators of cement samples under cyclic compression. "Adhesor Fine" zinc phosphate cement had the lowest duration. The longest was the sample of zinc-phosphate cement "Unitsem", glass ionomer cements "Cemion F" and "Fuji I" have satisfactory performance, slightly lower performance in polycarboxylate cements "Belokor" and "Adhesor Carbofine" (Table II).

A clear idea of the cyclic strength of the tested samples is given by the so-called Wehler curves – the dependence of the amplitude load on the number of cycles N that the sample withstood under a given load.

In double logarithmic coordinates, such dependences, as a rule, have a linear character, while, naturally, with a decrease in the load, the number of cycles to failure increases.

Specimen duration is determined by properties such as cement strength, adhesion of cement to tooth and artificial crown.

## DISCUSSION

Comparative analysis of these properties shows that the greatest duration, that is, the best functional properties are those cements that, on the one hand, do not exhibit brittle properties, and, on the other hand, have the best adhesive characteristics[5].

Failure of model specimens under cyclic loading can occur due to the loss of bond between the cement and the artificial crown or tooth, as well as a result of the destruction of cement.

For brittle cements, fracture is possible even at low loads and a small number of loading cycles due to possible large overvoltages near inevitable defects in the cement layer (cracks, cavities, etc.).

For cements that are plastic, this hazard can be largely reduced by plastic relaxation, which relieves the specified overstress and provides a longer duration.

## CONCLUSIONS

The results obtained for the duration of cement samples under cyclic compression showed that glass ionomer cement "Fuji I" has the highest value ($9 \times 10^5$H), while "Adhesor Fine" has the lowest ($3,5 \times 10^5$H), which is obviously related to their chemical composition, while polycarboxylate and some glass ionomer cements are intermediate. This most clearly simulates a real situation and will allow you to choose the necessary cement for fixing fixed structures of dentures.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

# DNA SEQUENCING OF NOVEL YEAST ISOLATED FROM BLOODSTREAM INFECTIONS IN AL-NAJAF PROVINCE

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**ABSTRACT**
**The aim**: To identify of fungal isolates using PCR techniques with universal primer (ITS1-ITS4 and ITS3-ITS4). A total of 533 blood samples from cancer patients, renal failure patients and patients who underwent cardiac catheterization have been included in this study.
**Materials and methods**: Devices and tools were used to preceding the study as shown in table (I), while biological and chemical materials are given in table (II).
**Results**: Three groups, 44 isolates of *Candidaspp* were isolated: 35(79.6%) isolates from cancer patients, 6(13.6%) isolates from patients with renal failure, and 3 (6.8%) isolates from patients with cardiac catheterization. These yeasts were diagnosed by conventional methods and by CHROMagar Candida medium, as well as by molecular methods to detect the regions of ITS2, ITS1, and the isolates were diagnosed as belonging to the yeast Candida spp. These isolates were also diagnosed using DNA sequencing detection technology and 12 new strains were recorded for the first time In the name of the researcher by the Japan Gene Bank.
**Conclusions**: It was concluded that there was high susceptibility of the polymerase chain reaction technique based on ITS 1, ITS2 primers in diagnosing the types of yeasts isolated from the bloodstream with high accuracy and speed compared to other traditional methods. Therefore, the DNA sequencing method is considered one of the best rapid standard methods for the diagnosis of fungi.

**KEY WORDS:** ITS regions, *Candida spp.*, DNA sequencing, bloodstream infection

## INTRODUCTION

Diseases caused by fungi and yeasts are known as fungal infections, and they are usually chronic diseases because the fungi grow slowly, as well as the difficulty of treating them because they are eukaryotes, which made them of great medical importance [1]. In normal conditions, fungi are less pathogenic and have a low ability to invade the body, but when the host's immune mechanism is weakened due to diseases or the use of immunosuppressive drugs, they will find the appropriate opportunity to invade the body and cause diseases. Although these fungi are of less importance than bacteria and viruses, they are opportunistic and have become It is of increasing importance, especially for patients with cancer, immunodeficiency syndrome, renal failure, and those taking immunosuppressive drugs [2]. Pathogenic fungi have the ability to cause severe damage to humans due to the secondary metabolic compounds they produce such as enzymes and toxins. In most cases, it is difficult to control fungal infections because they need specialized antifungal, in addition to the lack of vaccines against fungi, and the indiscriminate use of antifungal has led to the emergence of pathogenic strains. Resistance to antifungal due to fungi possessing genetic mechanisms of resistance, or a change in the permeability barrier or target site, and all of these methods are encoded by genetic factors that fungi possess [3]. The incidence of fungal bloodstream infections caused by pathogens such as Candida

spp. has increased in recent years, especially in intensive care units (ICUs). Candida bloodstream infections have been reported to be the fourth highest in-hospital infection. [4].Studies has shown that the incidence of fungal bloodstream infections in ICUs is 0.22–4.1% in developing countries and 0.024–0.687% in developed countries [5]. These rates appear to coincide with more widespread use of broad-spectrum antifungal drugs, Glucocorticoids, and immunosuppressive agents, as well as the low immunity of ICU patients and use of central venous catheter technology and other invasive procedures; however, early symptoms of fungal bloodstream infection lack specificity and low culture-positive rates can lead to a misdiagnosis [6]. Bloodstream fungal infections constitute a serious health problem because of the excessive hospital stay, added health care costs, and high morbidity and mortality attributed to the diseases. *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *Cryptococcus neoformans* are the most common yeasts causing bloodstream infections. These six species may account for 95 to 98% of all blood yeasts. *C. guilliermondii* and other, minor species maybe isolated occasionally [7]. Candidiasis is a parasitic fungal infection in or on any part of the body, such as fungi, caused by any of the species of Candida species, among which Candida albicans is the most common causative species. The infection is technically referred to as candidosis. These infections are broad-spectrum, ranging from

**Table I.** Devices and Tools used to preceding this study

| No. | Devices and Tools | Company name |
| --- | --- | --- |
| 1 | Agarose gel tank | Company/China |
| 2 | Autoclave | Harayma (Japan) |
| 3 | Bunsen burner | Iraqi |
| 4 | Centrifuge | Heraeus (England) |
| 5 | Compound light microscope | Olympus/Japan |
| 6 | Disposable Petri dishes | BBL- USA |
| 7 | Electrophoresis apparatus | Mupid-one (Japan) |
| 8 | Eppendorf centrifuge | Hettiich EBA.20/Germany |
| 9 | Eppendorf tube | Sigma/England |
| 10 | Gel electrophoresis unit | LKB/Swden |
| 11 | Incubator | Memmert , Germany |
| 12 | Micro pipettes | Gillson instruments/France |
| 13 | Nano drop | Labnet/ Germany |
| 14 | Slides and cover slides | BBL-USA |
| 15 | Standard loop 0.01 | Himedia(India) |
| 16 | Sterile cotton swabs | Himedia(India) |
| 17 | Vortex mixture | Buchi (Germany) |

superficial oral thrush and vaginitis to the deeply cited, often life-threatening systemic infection. Systemic infections are commonly referred to as candidemia and are one of the prominent co-infections in immunocompromised patients such as those suffering from cancer, HIV, renal replacement therapy, central-venous catheter (CVC) use (prolonged catheterization, type of catheter material, and anatomical site of catheter insertion), and healthcare practice (weak barrier methods during catheter insertion as well as patients in emergency surgery ) with extensive use of powerful antibiotics and immune-suppressive therapies during organ transplant or anti-leukemia therapies [8]. Molecular identification has become an important approach, with sequences in rRNA genes and spacers being used in DNA sequence analysis, microarrays or peptide nucleic acid probe based fluorescent hybridization assays. Non-culture based approaches to the diagnosis of yeast infections have been suggested as ways to improve the sensitivity and speed of a diagnosis, though currently few have been proved as sufficient and reliable alternatives to culture. [9]The term DNA sequencing refers to methods for determining order DNA nucleotide bases, knowledge of DNA sequences of genes and other parts of organisms' genomes have become indispensable to basics research in biological processes, as well as in areas such as diagnostics or forensic research [10]. Based on the information available in Iraq, there is little data available associated with fungal species isolated from bloodstream infections, and due to the increase in the number of immunosuppressed patients made many opportunistic yeasts more virulent and caused many cases of severe bloodstream infections, which are considered life-threatening for these groups of patients. The genetics of these yeasts may therefore be re-

considered in the strategies used in the treatment of these patients for these reasons , this study was designed to meet these spaces, this study confirms the importance of fungi as human causative agents and gives them Genetic and molecular profile for accurate identification of pathogens. Therefore, the current study aimed to find a database of fungi species that cause bloodstream infections to give their genetic characteristics and compared with standard isolates documented in the NCBI global sites. And to monitor and measure the occurrence of bloodstream infections in hospitals for immunocompromised patients and the detection of the most common pathogen among the cases of infection, this was achieved through the following objectives:

- Isolation and identification of fungal species from the blood of immunocompetent patients by microscopic examination and the formation of the germ tube and growth on CHROMagar medium etc.
- Identification of fungal isolates using PCR techniques with universal primer (ITS1-ITS4 and ITS3-ITS4).
- DNA sequencing analysis and aligned with NCBI database using Blast software and multiple aligned to each other using BioEdit software for identifying some fungal isolates.
- Registration of new strains and giving them an accession number by gene bank through the NCBI website.

## THE AIM
To identify of fungal isolates using PCR techniques with universal primer (ITS1-ITS4 and ITS3-ITS4). A total of 533 blood samples from cancer patients, renal failure patients and patients who underwent cardiac catheterization have been included in this study.

**Table II.** Biological and chemical materials were used in this study

| No. | biological and chemical materials | Company name |
|-----|-----------------------------------|--------------|
| 1 | Agarose | Sigma (USA) |
| 2 | De-ionized water | Bioneer /Korea |
| 3 | DNA Ladder | Promega/USA |
| 4 | DNA Loading Dye | Promega |
| 5 | EDTA | Promega/USA |
| 6 | Ethidium bromide | Sigma (USA) |
| 7 | Glycerol | Sigma (USA) |
| 8 | Gram stain kit | Himedia-India |
| 9 | Human serum | Iraqi |
| 10 | Lacto phenol cotton blue | Himedia-India |
| 11 | Lacto phenol cotton blue stain | Fluka / Switzerland |
| 12 | DNA Ladder | Promega/ USA |
| 13 | Master Mix | Promega / USA |
| 14 | Normal saline | Haidylena (Egypt) |
| 15 | Primers | Bioneer/ China |
| 16 | TBE buffer | Promega/USA |
| 17 | TE buffer | Promega/USA |

**Table III.** Three Readymade Culture media used in present study

| No. | Media | Company name |
|-----|-------|--------------|
| 1 | CHROM agar Candida | Himedia-India |
| 2 | Potato dextrose agar | Himedia-India |
| 3 | Sabouraud dextrose agar | Himedia-India |
| 4 | Brain heart infusion broth | Himedia-India |

**Table IV.** Specific primers used in this study and their Oligonucleotide sequences for Candida species detecting

| Primer | DNA sequences (5 `---3`) | Target region |
|--------|--------------------------|---------------|
| ITS3 F | 5 ´-GCA TCG ATG AAG AAC GCA GC-3' | ITS2 |
| ITS4 R | 5´-TCC TCC GCT TAT TGA TAT GC-3' | |
| ITS1 F | 5'-TCCGTAGGTGAACCTGCGG-3' | ITS1 |
| ITS4 R | 5´-TCC TCC GCT TAT TGA TAT GC-3' | |

## MATERIALS AND METHODS

## MATERIALS

### DEVICES AND TOOLS
The following devices and tools were used to preceding the study table (I)

### BIOLOGICAL AND CHEMICAL MATERIALS
The biological and chemical materials in table (II) were used for proceeding of experiments and tests in this study such as following:

### READYMADE CULTURE MEDIA
Three Culture media illustrated in table (III) were used as readymade for proceeding of experiments and tests in this study.

### PCR MATERIALS

### KITS
A specific EZ-10 spin column fungal genomic DNA mini-preps kit (Favorgen Biotech Corp, Taiwan), was used for purification of young yeast colonies.

### REACTION MIXTURE
Accu power PCR Pre Mix. Favorgen Biotech Corp, Taiwan, (0.1ml) thin-wall 8-strip tubes with attached cup /96 tubes were used.

### MOLECULAR WEIGHT MARKER
DNA marker Favorgen Biotech Corp, Taiwan, gene ruler, 100-1500bp, Kit size 50μl (0.1 μg/μl) were used.

### PRIMER
The following primers were used in the study for genome amplification [11], table (IV).

### PCR REACTION MIXTURE
The following components were mixing in the Thermal cycler to produce PCR products table (V)
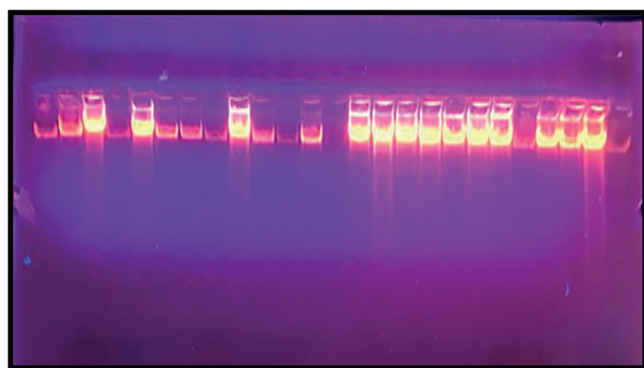
**Fig. 1.** Quantitative DNA concentration was measured using a nanophotometer
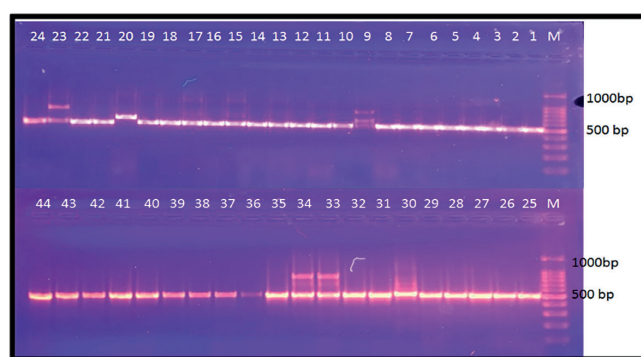


**Fig. 2.** Agarose gel electrophoresis of ITS1 regions PCR product by pair primers (ITS1-ITS4) of different yeast spp. (1.3g agarose gel, 80 volts for 1 hour) (M: DNA ladder; lane 1-44: yeast spp)

## PCR THERMO CYCLER CONDITIONS

### PCR PROGRAM

General program is listed in table (VI), using to amplification at target gene .

The PCR products and the ladder marker are analyzed by electrophoresis on 1.2 % agarose gel. The resolved band is indicative of the corresponding of (ITS2 and ITS1) genes. The molecular weight identification of resolved band is based on their correspondence to the ladder bands.

**Table V.** PCR mixture component used in the reaction

| PCR Master mix | Volume |
|---|---|
| DNA template | 5µl |
| Forward primer (10 pmol) | 1.5µl |
| Reveres primer (10 pmol) | 1.5µl |
| PCR water | 12µl |
| Total volume | 20µl |

## METHODS

533 blood samples were collected from hospitals in An-Najaf Governorate from patients undergoing catheterization 140 samples (26.27%), cancer patients 269 (50.47%) and patients with renal failure 124 samples (23.26%) during the period from 15 August 2020 to 21 March 2021. Data was retrieved from patient data register, individual patient records bloodstream infection was defined as the isolation of a micro-organism from bloodstream of patients with or without systemic symptoms was drawn under sterile conditions by venipuncture from a site not previously pricked. Two ml of blood was transported to the mycology laboratory in faculty of sciences in university of Kufa, and this blood specimens was immediately added to bottles containing 20ml of brain heart infusion broth and The bottles were incubated at 37ºC for up to five days. Subcultures were made on PDA and SDA agar plates after 5 days or in between if visible turbidity appeared, also CHROMagar Candida medium was performed and incubated at 37C° for 1-3 days for visible growth of Candida *spp* colonies and molds [12].

**Table VI.** PCR program that apply in the themocycler

| Gene Name | Temperature (ºC) / Time | | | | | Cycles Number |
|---|---|---|---|---|---|---|
| | Initial Denaturation | Cycling Conditions | | | Final Extension | |
| | | Denaturation | Annealing | Extension | | |
| ITS2 | 95/5 Min | 94/30 sec | 56/1min | 72/1min | 72/5min | 30 |
| ITS1 | 95/5 Min | 94/30 sec | 56/1min | 72/1min | 72/5min | 30 |

**Table VII.** Number of samples and isolates from the three categories of patients

| Species / Patients | NO. of samples | No. of isolates | C. parapsilosis | C. krusie | C. albicans | C. tropicals |
|---|---|---|---|---|---|---|
| Cancer | 269 | 35 | 15 | 9 | 5 | 6 |
| Cardiac catheterization | 140 | 3 | 0 | 2 | 1 | 0 |
| Renal failure | 124 | 6 | 4 | 0 | 2 | 0 |
| Total | 533 | 44 | 19 | 11 | 8 | 6 |

**Fig. 3.** Agarose gel electrophoresis of ITS2 regions PCR product by pair primers (ITS3-ITS4) of different yeast spp. (1.3g agarose gel, 80 volts for 1 hour) (M: DNA ladder; lane 1-44: yeast spp).

## PREPARATION OF CULTURE MEDIA

Four Media used in this study listed in Table (II, III). All media were prepared according to the manufactures fixed on their containers.

## YEAST ISOLATES IDENTIFICATION

All yeast isolates were diagnosed depending on the culture characteristics, microscopic features, biochemical test and molecular characteristics.

## PCR ASSAY

The PCR assay was performed to amplify 16S rDNA sequence for identification of *Candida spp.* using universal primers table (IV) for species identification.

## GENE AMPLIFICATION

Amplification of genes was carried out according to the experimental protocol of Accu power TLA PCR Premix tub under conditions of cycling as mentioned in cycling parameters of table (VI). The PCR reaction mixture was prepared as 5µl of Favorgen Biotech Corp, Taiwan, which is supplied in a ready to use in 100 µl PCR tube, followed by 5 µl temple DNA extract, 2µl of 10 pmol/µl upstream (reveres) primer specific solution as mentioned in table (V), µl of 10 pmol/µl downstream (forward) primer specific solution and the volume was completed to 20 µl with deionized distilled water, the tube were mixed with vortex to dissolved the lyophilized blue pellet, and briefly spin down.

## DETECTION OF DNA CONTENT BY AGAROSE GEL ELECTROPHORUS

Gel electrophorus was used for detection of DNA by UV transuliminator.

## AGAROSE PREPARATION

To prepare 100 ml of agarose solution, 1.8g of agarose was added to 100 ml(1X) TBE buffer into a glass flask,
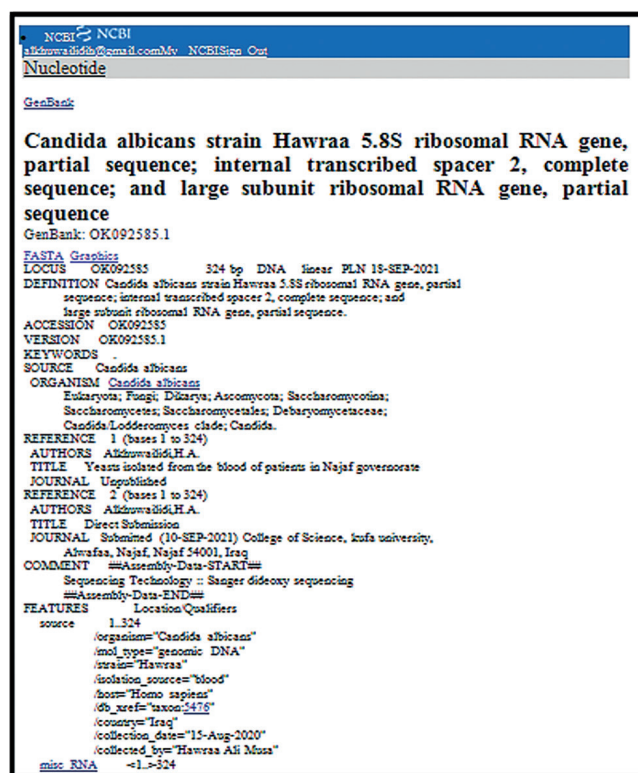


**Fig. 4.** Report of Candida albicans strain

then leave to boiling in a microwave, followed by cooling at 55ºC,then 5µl of ethidium bromide is added to agarose and poured on preparing tray. Comb was removed after hardening of agarose leaving wells which are used later to put DNA samples [13].

## SAMPLE PREPARATION

About 5 µl of DNA sample was placed in each well in agarose gel.

## AGAROSE ELECTROPHORESIS

TBE (1X) buffer was poured to the electrophoresis tank; tray with agarose gel was immersed in electrophoresis tank. Each well is loaded with 5µl of DNA sample and standard molecular weight of DNA ladder (marker) is loaded in a first well. Electrophoresis run at 80 volts for 1hr, gel was visualized under UV transuliminator and photographed by using digital camera [14].

## DNA SEQUENCING OF PCR PRODUCTS

The PCR products of *Candidaspp* were sent to Macrogen Lab in USA and received the data of sequences for every *Candida* spp which sent until sequencing reaction, purified PCR product with processor kit (Promega, Madison, USA) for PCR purification according to industrialization company. Subjected the sequencing results for multi-alignment setup on BioEdit software at finely phylogeny tree is commands and the multigene are perform via Mega 6

software. The sequencing results of the PCR products of several SSCP patterns were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The primary protein structure building of each SSCP genotype was begin by mutating the available reference NCBI DNA sequences of each coding genetic fragment, by substituting each observed SNP from the BioEdit/Laser gene software into its accurate position in the whole reference DNA sequence to represent each altered variant.

## RESULTS AND DISCUSSION

Depending on the table (I) the current study included the collection of 533 specimens from three groups of patients: cancer patients, patients with renal failure and patients with cardiac catheterization, only 44 samples gave positive growth results, the results were distributed as follows: 35 positive samples from cancer patients, 3 samples from patients undergoing cardiac catheterization, and 6 samples from patients with renal failure, in addition to the 39 control samples that were taken from healthy people, all gave negative results. The blood samples were grown on BHI broth from two to seven days at a temperature of 37 Cᵒ, and by observing the turbidity of the culture medium, Gram stain test was carried out for all positive isolates, and all of them gave a positive result of this test. Non-albicans Candida (NAC) constituted the majority of isolates, and *C. parapsilosis* was the most frequent in terms of NAC isolates. these results agreed with [15] Where they found that the group Candida nonalbicans is the main reason for the appearance of invasive Candida was heading upwards overtime while *Candida albicans* is decreasing, while disagreed with [16]were the most frequent from *Candida albicans,* and the study showed a remarkable high rate of fungal infection in a group of cancer patients relative to the other groups under study, where the number of fungal infections in this group was 35 out of a total of 269 patients due to the use of chemotherapy and radiation for these patients, which weakens their immune system significantly, which it makes them more susceptible to infection with various pathogens, especially fungi, which constitute a large proportion of the normal flora on the human body and its digestive canal, which is opportunistic in those cases (table VII).

Molecular diagnosis of isolated yeasts was carried out using total chromosomal DNA with PCR technology to identify the isolates, chromosomal DNA was extracted from Isolates using extraction kit (Favorgen Biotech Corp, Taiwan) according to Manufacturer's instructions. DNA concentration was measured Quantification using a nanophotometer and DNA purity was determined in the range of 1.7-2.0 figure (1).

The results showed that our different molecular sizes of the ITS region are *Candida spp*. Primers was used for the same sample, is (ITS1-ITS2) ITS regions have been targeted It was followed according to Key [18], PCR

products of these isolates in figures (2) and (3).

PCR products were sent to Macrogen Lab in USA for sequencing analysis, (forward and reverse strand and our sequences were compared with reference global sequences in national center biotechnology information (NCBI) Gene Bank and strains was received online and aligned to NCBI database using blast software and multiple aligned to each other, using Bio Edit software and submitted in fast a format to NCBI through sequin software. Online NCBI blast software was used to compare resulting sequences to the NCBI .The diagnosis of most of the isolates using PCR technique showed agreement with the diagnosis by DNA sequencing technique, and 12 new strains were first named after the researcher Hawraa by the Japanese Gene Bank according to the reports of the NCBI website, Each isolate has been given its own accession number as follows:

OK090878,OK090877,OK090876,OK090875,OK090874,OK090873,OK090872,OK090871,OK090870,OK090869,OK092585,OK083693, as in the report below (fig 4).

Through the current study, it was found that most of the yeast isolates were isolated from cancer patients after undergoing chemotherapy, as their weak immunity and their long stay in the hospital for the purpose of receiving treatment made them highly susceptible to infections due to their exposure to nosocomial infections, also yeast infections can occur in women who take steroids or antibiotics to treat or prevent bacterial infections, and the patient may succeed in overcoming cancer. But he dies due to a fungal infection. This results agree with [19] When collecting patient data at the Oncology Center, it was noted that the most prevalent type of cancer is breast cancer in women, followed by leukemia, followed other types of cancer when the isolates were diagnosed by biochemical tests, and the results were confirmed by culturing them with CHROMagar Candida Medium, the current study showed the high susceptibility of the polymerase chain reaction technique based on ITS 1, ITS2 primers in diagnosing the types of yeasts isolated from the bloodstream with high accuracy and speed compared to other traditional methods and The DNA sequencing method is considered one of the best rapid standard methods for the diagnosis of fungi.

## CONCLUSIONS

It was concluded that there was high susceptibility of the polymerase chain reaction technique based on ITS 1, ITS2 primers in diagnosing the types of yeasts isolated from the bloodstream with high accuracy and speed compared to other traditional methods. Therefore, the DNA sequencing method is considered one of the best rapid standard methods for the diagnosis of fungi.

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ORIGINAL ARTICLE

# FEATURES OF COVID-19 PNEUMONIA DIAGNOSIS

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**ABSTRACT**
**The aim:** The purpose of the study is to evaluate the clinical and laboratory features of COVID-19 pneumonia course, the diagnostic significance of laboratory methods for detecting the SARS-CoV-2 virus based on a retrospective analysis.
**Materials and methods:** We studied the case histories of 96 patients who were treated at the Municipal Non-Profit Enterprise "Lviv Clinical Emergency Care Hospital" for the period from 01/07/2020 to 31/07/2020 with a diagnosis of pneumonia, which corresponded to 5 points on the CO-RADS scale. We analyzed the clinical and laboratory signs of COVID-19 pneumonia depending on the results of the Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) tests to the SARS-CoV-2 infection (positive result of RT-qPCR was observed in the first group and negative – in the second group).
**Results:** In both groups, no clinical differences in the course of the disease were found. The most common symptoms of coronavirus pneumonia were found with the same frequency in both patients with a laboratory-confirmed diagnosis and without it. A positive PCR test in nasopharyngeal and oropharyngeal swabs was more often detected during testing up to 10 days, in patients over 60 years of age and in severe COVID-19.
**Conclusions:** The COVID-19 pneumonia diagnosis should be based on a combination of clinical, laboratory, and radiological signs of this disease. A negative PCR test result does not exclude the diagnosis of coronavirus disease. The test results are influenced by the timing of the sampling, the severity of the disease and the age of the patients.

**KEY WORDS:** COVID-19 pneumonia, Quantitative Reverse Transcription Polymerase Chain Reaction, computed tomography scan, CO-RADS scale

## INTRODUCTION
The COVID-19 pandemic is a new disease that has engulfed humanity and fostered the efforts of the entire global health community to solve a monumental challenge – to establish mechanisms for the diagnosis, treatment and prevention of this disease. According to the recommendations of the World Health Organization (WHO), the European Centre for Disease Prevention and Control (ECDC), the Agency for Health Technology Assessment and Tariff System (AOTMiT) and other leading health organizations, the main methodology used to diagnose SARS-CoV-2 infection is molecular technique that reveals the genetic material of the virus in the exudates and secretions of the patient, especially from the mucous membranes of the throat and nose [1, 2]. Taking into account the speed of testing and availability of equipment, one of the best methods for detecting viral RNA is the Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) [1].

At the same time, the results of recent studies indicate that the sensitivity of the RT-qPCR method for confirming COVID-19 is 60-71% [3-7], and therefore some patients with symptoms of the disease remain without an established etiological diagnosis. The underlying causes of such sensitivity of the polymerase chain reaction (PCR) may be associated with the insensitivity of test systems to the detec-

tion of nucleic acids due to virus mutations, low initial viral load in various body environments, or improper clinical sampling [8]. At a certain stage of the disease, samples from the lower respiratory tract (LRT) can be better diagnostic material than samples from the upper respiratory tract (URT), as is the case with MERS-CoV [8-10].

The detection of specific antibodies complements the PCR method for diagnosing the SARS-CoV-2 virus. Acute IgM antibodies appear only on the seventh day after contact with the pathogen. According to the available data, testing of specific antibodies against SARS-CoV-2 allows to achieve sensitivity at a level similar to that of RT-qPCR only 2-3 weeks after infection, i.e. when in some patients viral RNA is no longer detected, which is equivalent to the absence of infection [1].

Changes in the chest computed tomography (CT) are considered as a very important strategy for additional diagnosis of COVID-19, given the limitations of PCR and IFA methods, in particular, due to cases of false-negative results [11]. Interesting from a scientific and practical point of view are the results of a retrospective analysis, which compared the coefficient of sensitivity to RT-qPCR test and CT scan in 51 patients who had a burdened epidemiological anamnesis and manifestations of acute respiratory syndrome and/or fever. They consistently underwent CT

examination of the chest organs and RT-qPCR test, with an interval of one day – in the case of the first negative result, until a positive test result. The conclusions of this analysis showed that the sensitivity of the RT-qPCR test of the first swab from the oropharynx and nasopharynx was low and amounted to 70%, after the second test it increased by 24% (94%) and by another 3.9% after the third test (98%) while the sensitivity of CT screening in the viral pneumonia diagnosis was 98% [8]. The authors of this study stated that CT is a more sensitive method for diagnosing COVID-19 pneumonia than PCR. In another, more massive, report, the correlation between CT and RT-qPCR COVID-19 testing was carried out taking into account 1014 cases, which were included in the report. According to the data presented, only 59% of the examined patients had positive results of RT-qPCR test, while 88% showed changes on chest CT. In 75% of patients with negative RT-qPCR test results, there were signs of respiratory tract damage on CT; of them – in 48% of patients, the diagnosis of COVID-19 was considered extremely probable, in 33% – probable. Of the 1014 patients, 60% to 93% had initial positive CT scans consistent with COVID-19 before the initial positive RT-PCR results [12].

Overall, a systematic review with meta-analysis of COVID-19 diagnostic tests accuracy showed that CT has a high sensitivity (91.9% [89.8% -93.7%]) [11, 12]. The standardized assessment scheme for patients suspected of being infected with COVID-19 coronaviruses based on CT scans on the CO-RADS scale (COVID-19 Reporting and Data System) proposed by the COVID-19 Standardized Reporting Working Group of the Dutch Radiological Society makes it possible to assess the likelihood of coronavirus disease from very low – CO-RADS 1 to very high – CO-RADS 5 based on a typical X-ray picture – diffuse areas of "ground-glass" opacification and superimposed consolidations [13], which makes it an effective method in the COVID-19 diagnosis.

The object of this paper is to assess the clinical and laboratory features of the COVID 19 pneumonia course, the diagnostic significance of laboratory methods for detecting the SARS-CoV-2 virus based on retrospective analysis.

## THE AIM

The purpose of the research is to evaluate the clinical and laboratory features of COVID-19 pneumonia course, the diagnostic significance of laboratory methods for detecting the SARS-CoV-2 virus based on a retrospective analysis.

## MATERIALS AND METHODS

We studied the case histories of 96 patients who were treated at the Municipal Non-Profit Enterprise "Lviv Clinical Emergency Care Hospital" for the period from 01/07/2020 to 31/07/2020 with a diagnosis of pneumonia.

The criteria for the inclusion of patients in the retrospective analysis were determined diagnosis of pneumonia verified on the basis of laboratory and instrumental research

methods: the detection of the SARS-CoV-2 virus genome in swabs from the nasopharynx and oropharynx by the RT-qPCR method and/or the detection of IgM antibodies to SARS-CoV-2 coronavirus and the level of suspicion of COVID-19 infection on the CO-RADS scale, which corresponds to 5 points; burdened epidemiological anamnesis.

Patients without a confirmed diagnosis of pneumonia, patients with a COVID-19 suspicion level on the CO-RADS scale of 1-4 points, patients with other etiological variants of pneumonia (bacterial, hypostatic) and pulmonary tuberculosis were not included in the analysis.

We assessed the following parameters: age, gender of patients, day of illness at the time of hospitalization, illness symptoms, presence of concomitant diseases. We paid attention to the results of physical examination, indicators of blood oxygen saturation, body temperature (T), blood pressure (BP) and heart rate (HR). We analyzed changes in peripheral blood, glycemic indicators.

In accordance with the order of the Ministry of Healthcare of Ukraine dated 11/11/2020 No. 2583 "On Amendments to the Protocol "Provision of Medical Care for the Treatment of Coronavirus Disease (COVID-19)", we assessed the pneumonia severity according to the following criteria: respiratory rate ≥30 / min (adults), oxygen saturation of blood ≤92%, PaO2 / FiO2 ratio <300, pulmonary infiltrates > 50% of the lung field, according to which one or more of the detected criteria corresponds to the severe disease.

Statistical data processing was carried out using the Statistica 6.0 software package.

## RESULTS

According to the results of the analysis, the percentage of patients with SARS-CoV-2 virus detected by RT-qPCR method and serological tests was only 46.9% among patients diagnosed with bilateral pneumonia CO-RADS 5 points.

The average age of the patients was 57.95 ± 1.6 (18-89) years. The percentage of patients over 40 years old was significantly higher – 86.5%. Among the patients, the proportion of women prevailed – 55.2%, men – 44.8%.

The average day of patient hospitalization corresponded to 9.9 ± 0.53 day of illness. The proportion of patients with late admission to hospital exceeded, after 10 days from symptom onset – 51.6%, admissions before 10 days of illness were noted in 43.2% of cases.

In hospitalized patients, the following disorders were noted: in 42.7% – shortness of breath, in 50% – cough, in 17.7% – general weakness, in 10.1% – a loss of smell was documented, in 8.3% – nausea and vomiting, 4.2% of patients complained of chest pain, in 2.1% the leading complaints were diarrhea and decreased appetite.

In 77.1% of patients, the temperature rise was measured from sub-febrile to febrile, in 64.6% – tachycardia was detected. In 55.2% of patients, a decrease in blood oxygen saturation of ≤95% was revealed.

Changes in peripheral blood corresponded to the following picture: the majority of patients (77.6%) demonstrated

**Table I.** Age, data of physical and laboratory examinations of patients with laboratory-confirmed (first group) and without laboratory-confirmed (second group) diagnosis of coronavirus disease.

| Indicators | 1 group M±Std.dev | 2 group M±Std.dev | P |
|---|---|---|---|
| Age, years | 60,63±11,87 | 61,76±14,26 | 0,731 |
| Saturation, % | 89,33±16,07 | 90,84±4,52 | 0,609 |
| SBP, mm Hg | 134,60±15,42 | 135,59±32,01 | 0,878 |
| Heart rate (HR), bpm | 91,56±4,45 | 89,12±14,54 | 0,471 |
| Body temperature (T), $^{o}$C | 37,9±0,74 | 38,13±0,82 | 0,298 |
| WBC, 109/L | 8,05±3,13 | 8,95±3,97 | 0,353 |
| RBC, 1012/L | 5,01±0,67 | 4,71±0,51 | 0,130 |
| PLT, 109/L | 260,78±96,1 | 304,25±83,1 | 0,131 |
| ESR, mm/h | 22,73±13,95 | 34,44±19,13 | 0,02 |
| Total fibrinogen, g/L | 5,43±1,92 | 5,83±0,95 | 0,749 |
| Blood sugar, mmol/L | 6,40±4,66 | 7,23±5,40 | 0,576 |

**Table II.** Gender, risk factors, clinical features of pneumonia in persons with laboratory-confirmed (first group) and without laboratory-confirmed (second group) diagnosis of coronavirus disease.

| Indicators | | Frequency, n | | Percentage, % | | P |
|---|---|---|---|---|---|---|
| | | 1group | 2 group | 1 group | 2 group | |
| Gender | Women | 24 | 29 | 58,5 | 53,7 | 0,726 |
| | Men | 16 | 25 | 39,0 | 46,3 | 0,646 |
| Age up to 60 years | | 14 | 24 | 34,1 | 44,4 | 0,533 |
| Testing up to the 10th day of illness | | 25 | 16 | 62,5 | 29,1 | 0,037 |
| Oxygen-Dependent Patients | | 10 | 6 | 24,4 | 11,1 | 0,515 |
| AH | | 25 | 16 | 61,0 | 29,6 | 0,05 |
| CAD | | 22 | 13 | 53,7 | 24,1 | 0,09 |
| DM | | 10 | 4 | 24,4 | 7,4 | 0,469 |
| Loss of smell | | 2 | 1 | 4,9 | 1,85 | 0,833 |
| Shortness of breath | | 21 | 20 | 51,2 | 37,1 | 0,364 |
| Cough | | 25 | 29 | 61,0 | 40,7 | 0,137 |
| Nausea, vomiting | | 5 | 2 | 11,1 | 3,9 | 0,764 |
| General weakness | | 11 | 7 | 24,4 | 13,7 | 0,582 |
| Diarrhea | | 2 | 0 | 4,4 | 0 | 0,805 |
| Chest pain | | 0 | 2 | 0 | 3,9 | 0,778 |

an acceleration of the erythrocyte sedimentation rate (ESR), in almost half of the cases (47.8%) – leukocytosis was diagnosed and 47.8% of patients had a normal level of leukocytes. In 4.5% of patients, leukopenia was noticed. In some cases (9.1%), thrombocytopenia was diagnosed.

The level of total fibrinogen (100%) was elevated in all patients who underwent such test. In 38.9% of patients, an increased glycaemia was detected before the initiation of treatment. In every seventh patient (14.8%), a decrease in sugar level below the limit norms was determined.

Most often, patients had concomitant diseases of the cardiovascular system, namely: in 42.7% of patients, arterial hypertension (AH) was diagnosed, in 36.5% – coronary artery disease (CAD), in 29.2% – heart failure (HF), in 3% – there were the heart rhythm disorders in the form of atrial fibrillation, in 1 case – dilatation of the heart chambers.

**Table III.** Influence of patient age, testing time and illness severity on PCR test assessment using Fisher's exact test with Yates' continuity correction.

| Indicators | Testing up to the 10th day of illness | Testing after the 10th day of illness | Age up to 60 years | Age over 60 years | Severe illness | Moderate illness |
|---|---|---|---|---|---|---|
| Positive PCR test | 25 | 15 | 14 | 27 | 26 | 6 |
| Negative PCR test | 16 | 34 | 30 | 24 | 19 | 16 |
| Chi-square (df=1) | 8,33 | p= 0,0039 | 4,30 | p=0,0382 | 5,51 | p= 0,0189 |
| V-square (df=1) | 8,24 | p= 0,0041 | 4,25 | p=0,0392 | 5,43 | p= 0,0198 |
| Yates corrected Chi-square | 7,15 | p= 0,0075 | 3,48 | p=0,0622 | 4,36 | p= 0,0369 |
| Phi-square | 0,09261 | | 0,04522 | | 0,08225 | |
| Fisher exact p, one-tailed | | p= 0,0036 | | p=0,0307 | | p= 0,0176 |
| two-tailed | | p= 0,0055 | | p=0,0610 | | p= 0,0220 |
| McNemar Chi-square (A/D) | 0,00 | p=1,0000 | 0,07 | p=0,7911 | 5,76 | p= 0,0164 |
| Chi-square (B/C) | 1,08 | p= 0,2976 | 2,13 | p=0,1443 | 1,93 | p= 0,1649 |

Type 2 diabetes mellitus (DM) was registered in every fifth patient, in 4.2% of cases – diabetes was revealed for the first time.

4.2% of patients suffered from chronic obstructive pulmonary disease (COPD) and the same number – of urinary system diseases (glomerulonephritis, urolithiasis and pyelonephritis).

Chronic rheumatic heart disease, adrenal adenoma, breast cancer (condition after mastectomy), stomach cancer (gastrectomy according to Billroth 1), colorectal cancer (condition after hemicolectomy), hypothyroidism, acute pancreatitis were verified in every one case.

All patients included in the analysis had radiological signs of pneumonia, which corresponded to 5 points on the CO-RADS scale – a very high degree of suspicion. The patients were divided into two groups. The first group included 45 patients with confirmed laboratory COVID-19 status, the second – 51 patients with pneumonia without laboratory confirmed COVID-19 status. Comparative characteristics of both groups are shown in Tables I and II.

As shown in Table I, age, physical and laboratory findings did not differ significantly in both patient groups. The only exceptions concerned ESR indicators, which were significantly higher in patients of group 2 without laboratory-confirmed COVID 19 status (p = 0.02).

In both groups analyzed, the proportion of men and women was de facto equal (p = 0.726). The percentage of patients under 60 years old in the 1st and 2nd groups also did not differ significantly (p = 0.184). The key difference that, in our opinion, influenced SARS-CoV-2 test results, was a smaller proportion of patients who underwent the test before the 10th day of illness in the second group of patients (p = 0.037).

In both groups, no clinical differences in the course of the disease were found. The main symptoms of coronavirus pneumonia were found with the same frequency both in patients with a laboratory-confirmed diagnosis and without it. AH (p = 0.05) and unreliable CAD (p =

0.09) were detected in patients with a PCR-positive test a little more often. In particular, the presence of a comorbid pathology, according to the treatment protocol, may affect the hospitalization terms of such patients.

For better clarity of the presented findings, we analyzed the dependence of positive PCR test results on patient age, testing time and COVID-19 pneumonia severity using Fisher's exact test with Yates' continuity correction. The results of this analysis are presented in Table III.

## DISCUSSION

A positive PCR test in nasopharyngeal and oropharyngeal swabs was more often detected during early testing (up to 10 days), in patients over 60 years of age and in severe COVID-19. Probably in these categories of patients, the virus replication was more active, which influenced the test results.

It is worth noting that the results of our analysis are consistent with data from cohort studies conducted and published earlier. In particular, the highest percentage (100%) of positive results of RT-qPCR test for the SARS-CoV-2 virus, according to the results of one of the original studies of 56 hospitalized patients with confirmed SARS-CoV-2 infection in hospital departments in Wuhan (China), was obtained only in the first week of illness. Accordingly, at 2, 3, 4, 5 and 6 weeks the frequency of positive tests was lower and corresponded to 89.3%, 66.1%, 32.1%, 5.4% and 0%. [3]. The findings of a retrospective cohort study of viral load dynamics assessment in 96 patients infected with SARS-CoV-2 in the Chinese province of Zhejiang in January-March 2020, published by authoritative British Medical Journal, are similar to those previously published, in particular, the SARS-CoV-2 detection frequency in the obtained samples from the respiratory tract decreases over time: from 95% at the 1st week from the onset of symptoms to 54% at the 4th week of illness with subsequent negative results [6].

In another leading study in the UK, in the first week after coronavirus disease symptom onset, the semi-quantitative viral load – geometric mean (GM) of the RT-PCR cycle threshold (Ct) was 28.18 (95% confidence interval (CI): 27.76–28.61); in the second week GM Ct was 30.65 (95% CI: 29.82–31.52; p < 0.001 compared with week 1) and after 14 days, GM Ct was 31.60 (95% CI: 31.60–34.49; p = 0.01 compared with week 1). RT-qPCR cycle threshold (Ct) values correlate strongly with cultivable virus. Thus, the ability to cultivate SARS-CoV-2 in patients with mild to moderate disease was highest in the first week and decreased significantly by day 10 after the onset of symptoms [14]. The strengths of this study include a relatively large number of analyzed patients – 754 persons that tested positive for SARS-CoV-2 by RT-PCR targeting the RNA-dependent RNA polymerase (RdRp) gene, inclusion of a large proportion (> 50%) of samples taken more than 7 days after symptom onset and that all analysis was performed in a single laboratory. Persistent SARS-CoV-2 replication was demonstrated in severe COVID-19 cases during longer period of time – up to 32 days after the onset of symptoms and even at high Ct values [14].

A meta-analysis of data from seven previously published studies providing results on RT-qPCR testing for SARS-CoV-2 (total n = 1330) showed: within 4 days – between infection (day 1) and the typical time of onset of symptoms (day 5), the probability of a false-negative result in an infected person decreases from 100% (95% CI, 100% to 100%) on day 1 to 67% (CI, 27% to 94%) on day 4. On the day of symptom onset (day 5), the median false-negative rate was 38% (CI, 18% to 65%). This decreased to 20% (CI, 12% to 30%) on day 8 and then began to increase again, from 21% (CI, 13% to 31%) on day 9 to 66% (CI, 54% to 77%) on day 21. Overall, in this pooled analysis, the rate of false-negative RT-qPCR tests was highest on the day of infection, and lowest at 8 days after infection, and then increased again [15].

The problem of diagnosing COVID-19 pneumonia we met, in our opinion, was primarily due to the untimely recourse of patients for medical help (more than 10 days) and late PCR testing, which significantly influenced the results of molecular laboratory diagnostics of the SARS-CoV-2 virus.

## CONCLUSIONS

The COVID-19 pneumonia diagnosis should be based on a combination of clinical, laboratory, and radiological signs of this disease. A negative PCR test result does not exclude the diagnosis of coronavirus disease, and in the presence of other signs of this disease, the approaches to treatment and prevention of Covid-19 should be identical as in patients with a positive PCR test. The test results are influenced by the timing of the sampling, the severity of the disease and the age of the patients, which must be taken into account when planning the sampling of nasopharyngeal and oropharyngeal specimens to verify the diagnosis of coronavirus disease.

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ORIGINAL ARTICLE

# CORRELATION BETWEEN RS6265 SNP IN BDNF AND THE CONTEXT OF DIABETES TYPE II INVOLVEMENT IN IRAQI PATIENTS

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**ABSTRACT**
**The aim**: In this study, we looked into the possible link between the G196A polymorphism in the BDNF gene and DM in Iraqi patients.
**Materials and methods**: By using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, 100 subjects were genotyped for the G196A SNP of the BDNF gene, 50 as DM and 50 as controls, age-sex and ethnically matched healthy controls. Analysis of covariance (ANCOVA) was used to assess the association of this polymorphism, and genotype frequencies were compared between patients and healthy controls.
**Results**: Our result show that patient with the AG (Val-Met) genotype had a 40%of total DM patients than those and GG (Val-Val) genotypes. Therefore, we concluded that as a future aspect of the report the work can be further extended on proteomic level wherein the corresponding change occurred due to the mutation in the protein can be further detected at structural and functional level.
**Conclusions**: conclusion of our result was any patient with covid-19 must need to follow up for at least 1 month after recovery to notified of the post-Covid symptoms especially the male gender

  **KEY WORDS**: Neurotrophin, BDNF, DM, rs6265, and HbA1C

## INTRODUCTION

The neurotrophin family contains growth factors that promote cell survival, differentiation, and death, and brain-derived neurotrophic factor (BDNF) is widely expressed in the adult mammalian brain [1]. It's important for the long-term survival, differentiation, and expansion of neurons during development, as well as the preservation of neural systems in adults [2]. Neurotrophin theory proposes that failure of neurogenesis and neural plasticity causes many psychiatric disorders such as schizophrenia and major depression, and BDNF is regarded to be one of the essential components in this theory [3]. They're made up of preforms that can be broken inside the cell to release mature, secreted ligands. Mature neurotrophins bind to the Trk family of receptor tyrosine kinases, promoting Trk-mediated differentiation or survival [4]. Proneurotrophins are not inactive precursors because they can be released and cleaved extracellularly and serve as high-affinity ligands for p75 NTR, which causes apoptosis in neurons and oligodendrocytes [5]. Each neurotrophin also activates the p75 neurotrophin receptor (p75NTR), which belongs to the tumor necrosis factor receptor superfamily. Neurotrophins activate Ras, phosphatidyl inositol-3 (PI3)-kinase, phospholipase C-1, and signaling pathways mediated by these proteins, such as MAP kinases, through Trk receptors [6]. In obese, non-insulin-dependent diabetic mice with a simultaneous decrease in body weight,

BDNF reduced nonfasting blood glucose levels without a significant reduction in food consumption per body weight. In the db/db mice model, which are identified by nonfunctional leptin receptors and provide a model for obesity and non-insulin-dependent T2DM, 3 weeks of intermittent BDNF injection dramatically lowered blood glucose concentrations and glycated hemoglobin (HbA1c) [7]. The nuclear factor-B (NF-B) and Jun kinase, as well as other signaling pathways, are activated when the p75NTR is activated [8].The gene that also goes by the name BDNF codes for the BDNF protein. This gene is found on chromosome 11 in humans. Val66Met (rs6265) is a single nucleotide polymorphism in the gene that causes a difference in valine and methionine at codon 66 due to adenine and guanine alleles [9].

## THE AIM

In this study, we looked into the possible link between the G196A polymorphism in the BDNF gene and DM in Iraqi patients.

## MATERIALS AND METHODS

Our study included 100 participants in age from 30 to 65 years old (mean age47.5, SD 9.6) and were divided into two
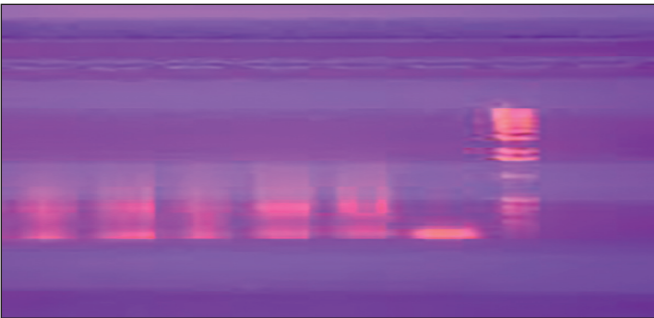
**Fig 1.** Genomic DNA Bands of Blood
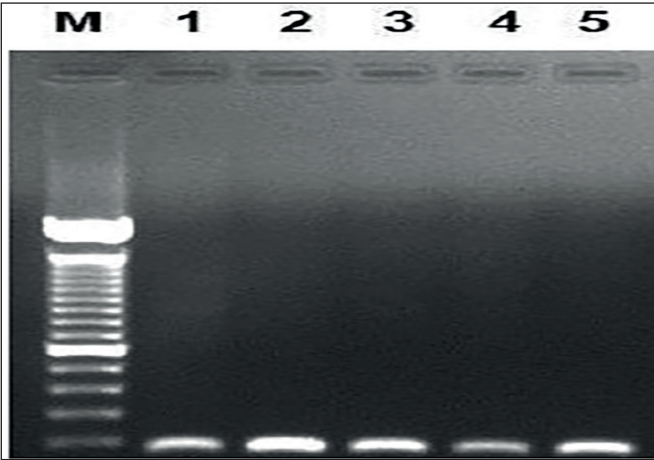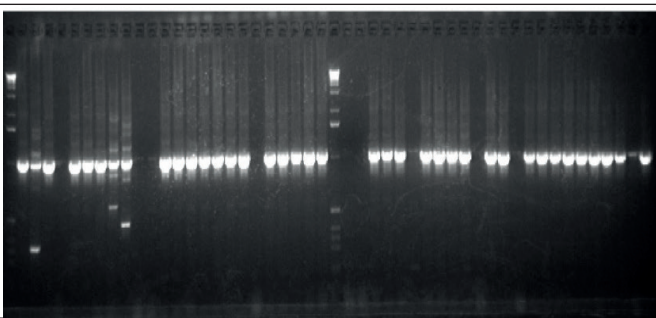


**Fig 2.** Amplicon Bands of Target region of BDNF
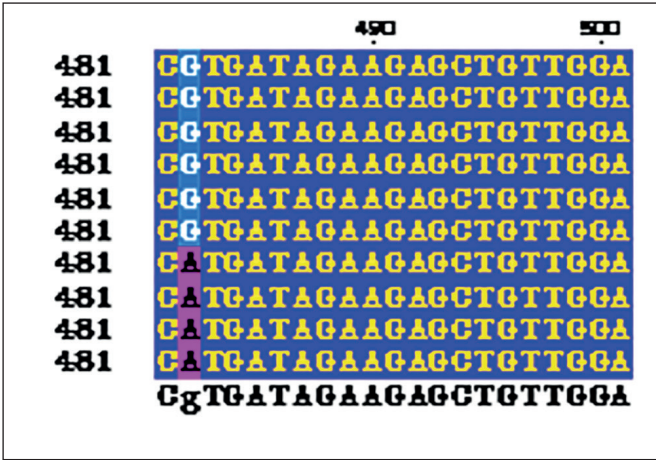


**Fig 3.** SNP obtained sequences by Tex Shade

groups: DM (50 patients) and control (50 participants). This study was conducted in Karbala City, the capital of Karbala Province, which is located in the heart of Iraq from December 2020 to August 2021.

## GENOTYPING

All participants had four milliliters of venous blood drawn into EDTA anticoagulant polypropylene tubes. All participants gave written agreement to be included in the study and were told about it. The Karbala University of Medical Science Ethical Committee evaluated and approved our protocol. A simple salting-out process was used to extract genomic DNA from patients' peripheral blood leukocytes [10]. A polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) approach was used to identify the BDNF Val-66Met polymorphism (dbSNP ID rs6265) at position G196A,

as previously reported.In brief, PCR was used to amplify the 272-bp region of the BDNF gene, which included the Val66Met polymorphism location. The restriction enzyme NlaIII was used to digest the PCR product overnight at 37 °C. Electrophoresis on an 8% polyacrylamide gel was used to identify the genotype, which was then stained with ethidium bromide and observed under ultraviolet light [11].

## ANALYTICAL STATISTICS

Analysis of covariance (ANCOVA) was used to assess the relationship between the Val66Met polymorphism between control and DM genotypesfrequencies between patients. We considered probability values of 0.05 or less to be statistically significant in this investigation. SPSS v. 26 was used for statistical analysis.

## RESULTS

Table (I) summarizes the baseline phenotypes and Val66Met genotypes of the participants in our study. The control group (normal HbA1C) and the patients were divided into two groups. Whenever the genotype frequencies of the Val66Met polymorphism were compared between these two groups, significant differences ($P < 0.05$) were found, table (II). Figure (1) was showed the Agarose Gel Electrophoresis of the DNA, figure (2) was showed that BDNF region Amplicons Bands, and figure (3) was showed Tex Shade of SNP sequences.

Agarose Gel Electrophoresis of Genomic DNA

**Table I.** Participants' baseline phenotypes and rs6265 (Val66Met) genotype distribution

| Phenotypes | SD | |
|---|---|---|
| Individual (n) DM group | 50 | |
| Age mean (years) | 47.5 | 9.6 |
| HbA1C mean (kg/m2) | 7.4 | 6.72 |
| Individual (n) control group | 50 | |
| Age mean (years) | 40.9 | 4.3 |
| HbA1C mean (kg/m2) | 5.2 | 3.2 |

**Table II.** DM Patients genotype frequencies

| Genotype | DM | | OR | 95% CI | | P value |
|---|---|---|---|---|---|---|
| | occur | Not occur | | Lower | Upper | |
| G/G | 2 (8%) | 23 (92%) | 0.005 | | | < 0.05 |
| G/A | 19 (95%) | 1 (5%) | | | | |
| cohort DM = Occur | | | 0.084 | 0.022 | 0.319 | |
| cohort DM = Not occur | | | 18.40 | 2.71 | 124.73 | |

## DISCUSSION

We investigated the putative link between the Val66Met (rs6265) polymorphism of the BDNF gene and DM in 40 patients in this study, and to our knowledge, this is the first study of its kind in Iraq. In this investigation, the Val66Met polymorphism was found to be strongly associated to DM, as well as DM with G/A (Met-Met) and GG (Val-Val) genotypes. DM is unquestionably exacerbated by metabolic and genetic variables, such as a lack of physical activity and the consumption of high-fat meals [12]. Differences in genetic makeup, on the other hand, may lead to variations in risk of DM in a group living in the same environment [13]. The BDNF gene encodes a neurotrophin family of growth factors that bind to the tyrosine kinase receptor tropomyosin-related kinase B (TrkB) and activate signaling [14]. It became pertinent to select and study such a gene that is common to DMT2 and Neurological disorder. BDNF was found to be one of such genes. Owing to this great significance of BDNF it was selected as target gene to be studied in terms of its SNPs [15]. A list of reported SNPs in BDNF were searched in dbSNP and one of them rs6265 at position 27,658,369 on chromosome 11 was selected for further studies. The aim was to check the frequency of this G to A Polymorphism in the test samples [16]. For this, the primer set was designed and amplification was performed on 100 diabetic samples. No control was taken as the purpose was to check the frequency of SNP in DMT2 samples only and not to compare with Non diabetic ones. The sequencing result when analyzed by Bioinformatics tool clearly indicated that four diabetic samples (Sample number 2, 3, 4 and 6) were found to have A instead of G (rs6265) at position (chr 11: 27,658,369) that corresponds to val66met polymorphism.

## CONCLUSIONS

The connection of the Val66Met polymorphism of the BDNF gene with DM as a DM trait was validated in the population of centralIraq in our study. Patient with the AG (Val-Met) genotype had a 40%of total DM patients than those and GG (Val-Val) genotypes.

## RECOMMENDATION

Further study on a wider and large sample set is recommended. As a future aspect of the report the work can be further extended on proteomic level wherein the corresponding change occurred due to the mutation in the protein can be further detected at structural and functional level.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

# COMPREHENSIVE STUDY OF MANIFESTATIONS OF BRAIN TISSUE RESOLUTION IN CASE OF VARIOUS TYPES OF STROKE

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**ABSTRACT**
**The aim:** The study is to research the resolution of perifocal brain tissue at various type strokes using immunomorphology
**Materials and methods:** The immunohistochemical study of perifocal brain tissue in 21 cases of various strokes types was conadcted
**Results:** When comparing the GFAP + astrocytes detection area at IS, HS and IS with HT, no significant difference was found. At the 1st degree of GFAP + astrocytes were in the border around the necrosis nucleus at IS and IS with HT, and at HS GFAP + astrocytes accumulated along the hematoma edge. CD34 + cells were found in most cases of strokes. Over time, cases with a larger CD34 + cells detection area increased (Kendal's Tau = 0.512, p = 0.001) in all groups. The capillary network at HS was around the hematoma and formed a gliomesodermal capsule with microglia and inflammation. 1st degree τ-protein accumulation was detected in 2/3 of cases (66.7%) of all strokes without significant difference. If compared in different stroke periods, τ-protein detection frequency increased and accumulated in brain structures – Kendal's Tau = 0.359; p = 0.023.
**Conclusions:** With the development of the disease, the number of cases with a larger area of detection of GFAP + astrocytes and CD34 + cells increased in strokes of various types. Τ-protein was detected in neurons in all variants of ACVA in the first period.

**KEY WORDS:** stroke, GFAP + astrocytes, CD34 + cells, τ-protein

## INTRODUCTION

Stroke is the leading cause of death and disability across the world. Mortality of those who have suffered a stroke is up to 40% during the first 12 months after the disease. Half of patients who have suffered an acute cerebrovascular accident (ACVA) lose their social adaptation and need outside help. Among all ACVAs, ischemic stroke (IS) is the most common type, making approximately 85% of the total number of ACVAs [1]. Hemorrhagic stroke (HS), in its turn, makes approximately 10-15% of all ACVAs [2]. About 50% of patients who have suffered HS fail to return to their previous place of work, and 95% of patients have cognitive disorders and low quality of life [3].

In case of IS, acute ischemia-reperfusion and excitotoxicity, and in case of HS, a mechanical pressure of the hematoma and the influence of hemoglobin degradation products upon the perifocal tissue of the brain [4] cause the increased permeability of the blood-brain barrier (BBB), vasogenic and cytotoxic cerebral edema. Consequently, it leads to the necrotic neurons [5], microglia and inflammation [6, 7]. Glial cells play an ambiguous role in a post-stroke period. On the one hand, they produce trophic factors and energetically support neurons, and on the other hand, they produce inflammatory mediators, stimulate inflammation and slow down healing [8, 9]. In case of acute cerebral ischemia, together with acute changes in the critical area of the brain due to ejection of proangiogenic growth factors [10] stimulation of angiogenesis and vasculogenesis takes place. Angiogenesis and vasculogenesis are considered the main processes making it possible to restore the integrity of connections between neuron, astrocyte and pericyte (vessel microcirculatory bloodstream) owing to remodeling and formation of new vessels after injury in the perifocal area of the brain [11].

Apart from acute changes in neurons, some of these cells, as a result of acute ischemia, and in case of HS under the influence of ferritin, there is hyperphosphorylation and accumulation of microtubule-associated τ-protein of neurons, with its accumulation in the cell as paired spiral filaments and neurophytes. [12]. These changes cause the loss of neural connections, which then clinically manifests itself as cognitive deficit [13]. As the problem of quality of life of patients who have suffered ACVA is up-to-date, in order to improve the diagnosis, prevention and treatment, pathomorphological changes of the critical area of the brain in case of strokes of various geneses, has been thoroughly studied.

## THE AIM

To study the manifestations of the resolution of the perifocal tissues of the brain in case of strokes of various types based on a comprehensive immunohistochemistry of neurons, astrocytic glia and angiogenesis depending on the period of the disease.

## MATERIALS AND METHODS

Samples of perifocal tissue of the brain of 21 deceased patients were taken for immunohistochemistry, of which 7 (33.33%) cases were IS, 8 (38.09%) were HS, and 6 (28.57%) were the cases of ischemic stroke with hemorrhagic transformation (IS with HT). The test material was grouped according to the classification of the stages of cerebral infarction Mena H et al. [14] and clinical and radiological classification of stages of organization of hematoma Bradley W. [15]. Based on these classifications, the time intervals corresponded to three periods: the first period – 1-3 days from the occurrence of stroke, the second – 4-7 days from the onset of the disease, and the third period > 7 days from the onset of ACVA (table I). The frequency of cases with various stages did not differ between groups (p>0.1, Mann-Whitney test).

In the course of the study, in each case, pieces of brain tissue 2.0x2.0 cm were sampled in the projection of the cortex and subcortical nuclei of the temporal lobe, which bordered on the area of necrosis or hematoma, then the brain tissue was fixed in 10% neutral formalin solution and dehydrated in alcohols with growing concentrations according to the standard method, poured into paraffin and as visual preparations stained sections with hematoxylin and eosin. For immunohistochemistry of neurons τ-protein marker was used (NeoMarkers, polyclonal), to study the state of astrocytic glia GFAP – glial fibrillar acidic protein (DAKO, polyclonal). To verify angiogenesis in the perifocal areas marker CD34 (DAKO, QBEnd10) marker was used. The expression of the antigens studied in the brain tissue appeared to be of a clear brown intracellular and extracellular staining in the course of the study under a light optical microscope Zeizz Primo Star (Germany) at a magnification of × 100, × 200 and × 400. Microphotographs were taken using a Leica DM 750/4 microscope (Germany) with a Leica DFC 420 digital camera (Germany) and Leica Application Suit Vers software 3.8. Immunohistochemistry was performed on the basis of the laboratory of the diagnostic and consulting center CSD Health care, Ukraine, Kyiv.

The work was approved by the Commission on Bioethics (excerpt from Protocol No.2 as of February 26, 2018), all moral, ethical and professional requirements and norms in the study of cadaver material were in line with the principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine and relevant laws of Ukraine.

Ranking scales were used to objectify and semiquantitatively compare the expression of the studied markers depending on the groups and periods of the disease. For the expression of GFAP + astrocytes and CD34 + cells, it was filling by them 0-30% (1st degree of distribution), 31-60% (2nd degree of distribution) and > 61% (3rd degree of distribution) of the field of vision. 9 fields of vision, which corresponded to an area of 1 mm$^2$ were assessed; and to increase the reliability of the assessment the average value of the detected changes in 9 fields of vision was used. For the accumulation of τ-protein, the scale included 4 gradations of the degree of distribution – none; presence only in neurons; neurons and interstitium; neurons, interstitium and astrocytes. Therefore, the degree was determined taking into account the worst of the results in 9 fields of vision.

The expression of the studied markers between groups was compared using the unpaired Mann-Whitney test. To assess the dynamics of expression according to the periods of the disease, the Kendall rank correlation coefficient (Kendal's Tau) was used, and pairwise comparison of periods was performed using the Mann-Whitney test. Statistical processing was performed using the software package STATISTICA for WINDOWS 6.0 (StatSoft, USA).

## RESULTS

### COMPARISON OF GFAP + ASTROCYTES BETWEEN ALL STROKE GROUPS

When comparing the area of detection of GFAP + astrocytes between all groups of strokes, no significant difference was identified (table I). In case of the 1st degree of GFAP expression +astrocytes were placed mainly in the border area around the necrosis nucleus, concentrated either around blood vessels or as single cells in the neuropile in case of IS and IS with HT, while in case of HS GFAP + astrocytes accumulated in brain tissue along the edge of the hematoma. There was a relative minority of such cases – 6 (28.6%, 25-33% depending on the group). To the contrary, most often in all groups there were cases with GFAP + cells of the 2nd degree of distribution: 12 (57.1%), and in HS and IS with HT groups they made the vast majority (62.5% and 66.7%, respectively). In such cases, GFAP + astrocytes distribution from the border area of the penumbra or perigematoma and were located diffusely, except for vessels, around hyperchromic neurons, neurons with vacuolated cytoplasm, and around neurons with chronic changes. GFAP + astrocytes, which in total occupied more than 60% (Figs. 1, 2) of the area, were detected in 2 (28.57%) cases in IS group and in 1 (12.5%) case in HS group.

### COMPARISON OF ANGIOGENESIS BETWEEN ALL GROUPS OF STROKE

Angiogenesis in the critical area with activated CD34 + cells was detected in most cases of strokes of all types (Table II). When comparing the severity of this feature between IS, HS and IS with HT no significant difference was identified, although this result may be caused by insufficient statistical sampling, as the proportion of cases with the 1st degree of angiogenesis (0-30%) in HS group was 62, 5% (Fig. 3), in IS with HT group it was -50%, and in IS group it was only 28.6%. To the contrary, the 3rd degree angiogenesis (> 61% of CD34 + cell distribution area) was detected only in IS group – 28.6% (marginal significance when compared with the sum of the other two groups, p = 0.1).
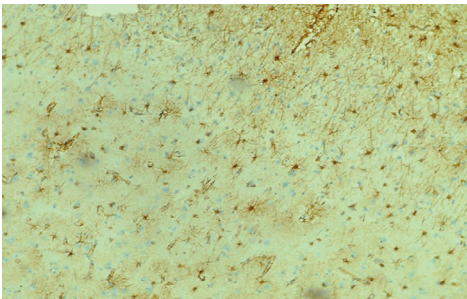
**Fig. 1.** Perifocal area of the brain with GFAP + astrocytes, some of which are elongated, with branched processes, occupying> 60% of the study area in case of ischemic stroke (IS).
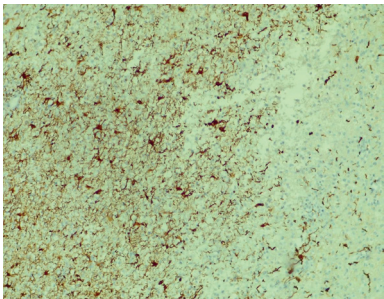


**Fig. 2.** GFAP +> 60% of the occupied area of perifocal tissue of the brain in case of hemorrhagic stroke (HS).
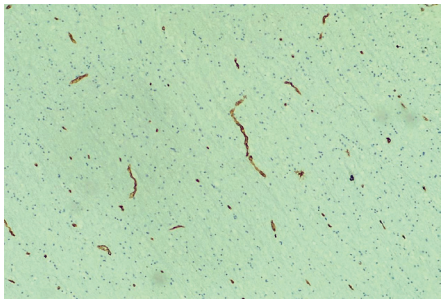


**Fig. 3.** Perifocal tissue of the brain with CD34 + cells that form single thin-walled capillaries and occupy up to 30% of the study area in case of ischemic stroke with hemorrhagic transformation (IS with HT).
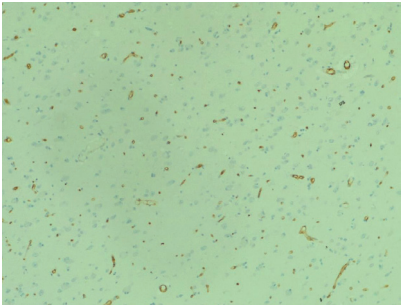


**Fig. 4.** CD34 + cells 0-30% of the occupied area of perifocal tissue of the brain in case of ischemic stroke (IS).
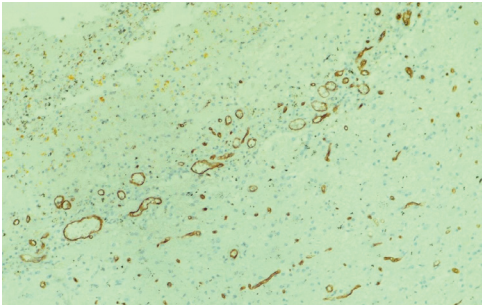


**Fig. 5.** CD34 + form a thin-walled densely located capillary network of the gliomesodermal capsule in the perigematous tissue of the brain in case of hemorrhagic stroke (HS).
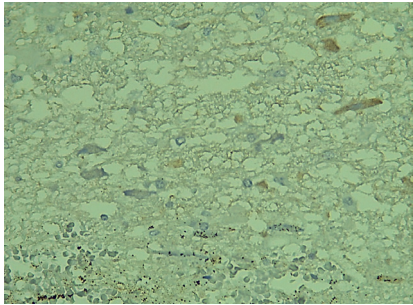


**Fig. 6.** Tau protein in neurons, neuropil and astrocytes in the perigematous tissue of the brain in case of hemorrhagic stroke (HS).
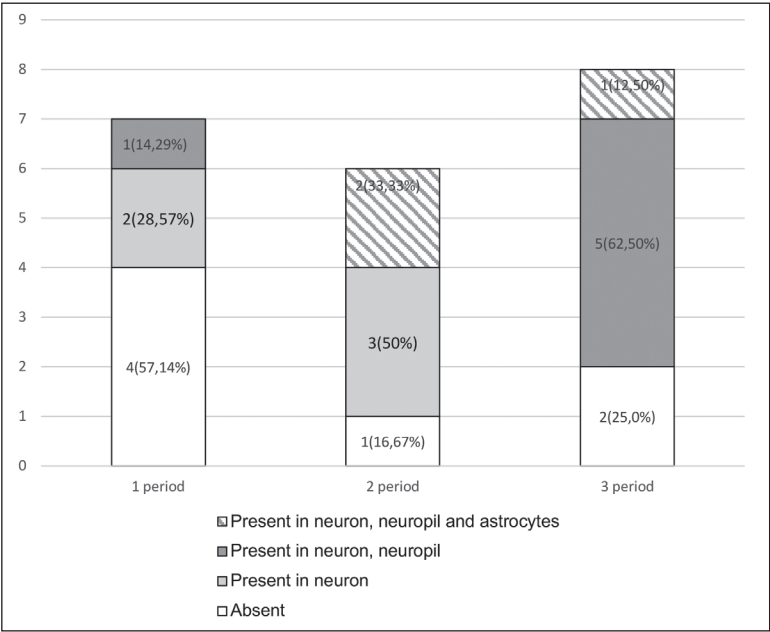


**Fig. 7.** Detection of τ-protein depending on the period of stroke. Notes: First period (1-3 days), Second period (4-7 days), Third period (> 7 days)

## COMPARISON OF T-PROTEIN DETECTION BETWEEN ALL STROKE GROUPS

In addition to 'red' and 'shadows' of neurons, neurons with chronic changes, activated microglia and newly formed vessels, immunohistochemistry of the perifocal tissue of the brain revealed acute hypoxia-stimulated processes of neurodegeneration with intracytoplasmic neuroprotective accumulation and hyperthyroidism. Accumulation of τ-protein of the 1st degree was detected in a total of 2/3 of cases (66.7%) of all stroke variants without significant differences between groups (Table II). The statistical sampling was insufficient to detect significant differences depending on the type of stroke in the

**Table I.** Distribution of the number of cases of strokes of various types by periods of the disease

| Periods of the disease | Ischemic stroke (n=7) | Hemorrhagic stroke (n=8) | Ischemic stroke with hemorrhagic transformation (n=6) |
|---|---|---|---|
| First period | 3 (42,86%) | 2 (25%) | 2 (33,33%) |
| Second period | 2 (28,57%) | 2 (25%) | 2 (33,33%) |
| Third period | 2 (28,57%) | 4 (50%) | 2 (33,33%) |

**Table II.** Damage markers (τ-protein) and resolution (GFAP + astrocytes and CD34 +) in various types of strokes: ischemic (IS), hemorrhagic (HS) and ischemic with hemorrhagic transformation (IS with HT).

| | IS (n=7) | HS (n=8) | IS with HT(n=6) | ***P(Mann-Whitney test) |
|---|---|---|---|---|
| *GFAP: n (%) | | | | |
| 0 degree (none) | 0 | 0 | 0 | |
| 1 degree | 2 (28,57%) | 2 (25%) | 2 (33,33%) | |
| 2 degree | 3 (42,86%) | 5 (62,50%) | 4 (66,67%) | NO |
| 3 degree | 2 (28,57%) | 1 (12,50%) | 0 | |
| *CD34: n (%) | | | | |
| 0 degree (none) | 0 | 0 | 0 | |
| 1 degree | 2 (28,57%) | 5 (62,50%) | 3 (50%) | |
| 2 degree | 3 (42,86%) | 3 (37,5%) | 3 (50%) | NO |
| 3 degree | 2 (28,57%) | 0 | 0 | |
| ** τ -protein: n (%) | | | | |
| 0 degree (none) | 3 (42,86%) | 2(25%) | 2 (33,33%) | |
| 1 degree | 1 (14,28%) | 2 (25%) | 2 (33,33%) | |
| 2 degree | 3 (42,86%) | 1 (12,50%) | 2 (33,33%) | NO |
| 3 degree | 0 | 3 (37,50%) | 0 | |

Notes:

n is the absolute number of cases; % – the relative number of cases

\* The value of the relative area of brain tissue with positive expression of the studied cell markers (GFAP and CD34): 1st degree – 0-30% of the area; 2nd degree – 31-60% of the area; 3rd degree -> 61% of the area

\*\* Presence of τ -protein in brain tissue structures: 0 degree – absent; 1st degree – in neurons; 2nd degree – in neurons and neuropil; 3rd degree – in neurons, neurops and astrocytes

\*\*\* No significant difference between the groups was found, p> 0.1 for all pairs of comparisons, the Mann-Whitney test.

severity of the accumulation of this protein – its presence in different structures (only neurons, neurons and neuropil; neurons, neuropil and astrocytes). However, in almost half of the cases – 3 (42.86%) in IS group the accumulation of pathological τ-protein was of the 2nd degree, in contrast when we talk about HS and IS with HT such cases made a third or less of the total number of each group. All cases with the presence of hyperphosphorylated τ-protein of the 3rd degree were detected only in HS group I – about 1/3 of the group – 3 (37.50%), but the difference becomes significant only if we compare HS group with other cases (Fisher's exact test).

## COMPARISON OF THE DETECTION OF GFAP + ASTROCYTES, CD34 + CELLS AND T-PROTEIN BETWEEN STROKE PERIODS

The assessment of the intensity of expression of markers

of injury and resolution of perifocal tissue of the brain (table III) in the temporal aspect revealed a clear increase in the expression of GFAP + astrocytes with the course of the disease – Kendal's Tau = 0.774, p <0.001. In the first period, GFAP + astrocytes were localized around the border zone with the area of 1st degree distribution, and 1 case of the 2nd degree. In contrast, in the second period in all cases of GFAP + astrocytes occupied the area of the 2nd degree distribution, and in the third period – in more than 1/3 of cases (37.50%) – expressed astrocytes showed an area> 61% of the studied portion of the brain, and in other cases – 31-60% of the studied brain tissue.

The critical area of the brain occupied by CD34 + cells in the first period, mainly did not go beyond the 1st degree of the entire perifocal area – 6 (85.71%) (Fig. 4), only in 1 (14.29%) case the detection area of CD34 + cells was 31-60%. In the second period, CD34 + cells created newly

**Table III.** Damage markers (τ-protein) and resolution (GFAP + and CD34 +) in different periods of stroke

| | First period (1-days) (n=7) | Second period (4-days) (n=6) | Third period (>7днів) (n=8) | R (Kendal's Tau), ***p |
|---|---|---|---|---|
| *GFAP: n (%) | | | | |
| 0 degree (none) | 0 | 0 | 0 | RGFAP =0,774 p <0,001 |
| 1 degree | 6(85,71%) | 0 | 0 | |
| 2 degree | 1(14,29%) | 6 (100%) | 5(62,50%) | |
| 3 degree | 0 | 0 | 3(37,50%) | |
| *CD34: n (%) | | | | |
| 0 degree (none) | 0 | 0 | 0 | R CD34=0,512 p=0,001 |
| 1 degree | 6(85,71%) | 2(33,33%) | 2(25%) | |
| 2 degree | 1(14,29%) | 4(66,67%) | 4(50%) | |
| 3 degree | 0 | 0 | 2(25%) | |
| ** τ -protein: n (%) | | | | |
| 0 degree (none) | 4(57,14%) | 1(16,67%) | 2(25,0%) | R τ =0,359 p=0,023 |
| 1 degree | 2(28,57%) | 3(50%) | 0 | |
| 2 degree | 1(14,29%) | 0 | 5(62,50%) | |
| 3 degree | 0 | 2(33,33%) | 1(12,50%) | |

n is the absolute number of cases; % – the relative number of cases

* The value of the relative area of brain tissue with positive expression of the studied cell markers (GFAP and CD34): 1st degree – 0-30% of the area; 2nd degree – 31-60% of the area; 3rd degree -> 61% of the area

** Presence of τ -protein in brain tissue structures: 0 degree – absent; 1stdegree – in neurons; 2nd degree – in neurons and neuropil; 3rd degree – in neurons, neuroples and astrocytes

**** Kendal's Tau.

formed thin-walled capillaries and in more than half of the cases – in 4 (66.67%) the area of distribution corresponded to the 2$^{nd}$ degree of distribution. In the third period, the intensity of angiogenesis increased: in half of the cases CD34 + cells (Fig. 5), which inhabited 31-60% of the study area were observed, and in 2 (25%) cases they occupied> 61% of the perifocal area. Only in ¼ of all cases of the third period the area of detection of CD34 + cells did not go beyond the 1$^{st}$ degree. Thus, as the disease develops, the number of cases with a larger area of detection of CD34 + cells naturally increases (Kendal's Tau = 0.512, p = 0.001).

Having compared changes in different periods of stroke, it has been identified that over time, the frequency of detection of τ-protein (from 42.86% in the first period and to 75% in the third period) increased, and it was accumulating in certain brain structures – Kendal's Tau = 0.359; p = 0.023.

In the first period of the disease hyperphosphorylated τ-protein of the 1$^{st}$ degree was found in 2 (28.7%) cases, in the second period it was found in 3 (50%) cases, also in the second period pathological τ-protein of the 3$^{rd}$ degree was observed in a third of cases (coming from the intracellular space of neurons, accumulated in neuropil and astrocytes). Over time, in the third period, hyperphosphorylated τ-protein of the 2$^{nd}$ degree was detected in more than half of the cases of strokes, and in one case this protein was diagnosed in all three studied structures of brain tissue (Fig. 6, 7).

Thus, all three study markers (GFAP +, CD34 +, τ-protein) showed a significant increase depending on the post-

stroke period, mainly due to the difference between the first period and the third period (p = 0.06 for τ-protein, p < 0.05 for the area of detection of GFAP + astrocytes and CD34 + cells, no significant difference between the second period and the third period was found, between the first period and the second period the difference was significant only for the area of detection of GFAP + astrocytes). Consequently, there was also a direct correlation between τ-protein and GFAP + (Kendal's Tau 0.484, p = 0.002) GFAP + and CD 34+ (0.740, p <0.001), but no correlation was found between τ-protein accumulation and CD34 + cell detection area. In the first period, it increased the most in all 3 types of strokes.

## DISCUSSION

Astrogliosis is a stereotypical reaction of astrocytes to brain injury [16 – 18]. In case of acute injury, activated astrocytes were located in the border area of the penumbra/perigematoma in some cases on the 2$^{nd}$ day from the onset of the disease. In subsequent post-stroke periods, in our study and according to other authors [19] as a compensatory-adaptive response, the frequency and area of detection of GFAP + astrocytes increased. Cells were located diffusely in the perifocal area, astrocytes were elongated, the processes of some of these cells went beyond their own domain, branched and intertwined with each other, establishing contacts with neurons and vessels, and

subsequently formed a glial scar. Post-stroke repair under the influence of growth factors [20 – 22] developed during the first 12-24 hours after stroke and lasted for 4 weeks. According to the results of our study early angiogenesis began with the germination of endothelial cells, the formation of tubular vascular branches and anastomoses in the perifocal parts of the brain. In all variants of ACVA, around the area of necrosis/hematoma, CD34 + cells were detected, the expression of which increased as the stroke continued [23, 24]. Less noticeable angiogenesis in case of IS with HT may be associated with secondary hemorrhage into the necrosis area. We believe that such changes are similar to the changes in case of HS, in which the rate of repair was influenced by the products of degradation of erythrocytes [25, 26]. In case of HS, a dense newly formed capillary network was located around the hematoma and in combination with microglia and inflammatory infiltrate separated the hematoma from the perifocal area and formed a gliomesodermal capsule.

In this study, τ-protein in brain neurons was observed in the acute period of stroke. This meant induced cell damage, destabilization of neuronal microtubules caused by impaired glutamate transport in the brain. The presence of this protein in the extracellular space after the neurons disappearance, its internalization by other brain cells, in particular astrocytes and distribution in the CNS 'contribute' to the pathogenesis of neurodegenerative proteinopathies, affecting both neurodegeneration [27 –29] and possibly neuroprotection, as well as act as potential mediators in the spread, and the elimination of protein-associated diseases [30].

## CONCLUSIONS

1. Early healing processes in the perifocal tissue of the brain began in the first three days of the disease, GFAP + astrocytes were located in the border area around the nucleus of necrosis/hematoma, and over time the intensity of GFAP + astrocytes increased, most in case of IS and IS with HT.
2. The least noticeable neoangiogenesis was found in IS with HT group. CD34 + cells in the acute period in case of HS were found in the area of the brain directly adjacent to the hematoma. CD34 + cells subsequently in combination with activated microglia and inflammatory cells became the basis for the formation of gliomesodermal capsule. With the development of the disease, the number of cases with a larger area of detection of CD34 + cells increased in strokes of various types.
3. Ţ-protein was detected in neurons in all variants of ACVA in the first period. With excessive hyperphosphorylation, in the second and third periods of stroke τ-protein came out of the periaxonal part of the neurons into the neurofield. In case of HS, this protein was captured and found in astrocytes, which may indicate the distribution of τ-protein between the structural components of the brain and subsequently clinically manifest itself in the disorder of the higher integrative function of the brain.

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ORIGINAL ARTICLE

# EFFECT OF 20-HOUR FASTING AND LOW FAT DIET ON GHRELIN HORMONE, GLUCOSE LEVEL AND LIVER FUNCTION IN ALBINO RATS MALE

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**ABSTRACT**
**The aim**: It aims to study the effect of fasting and low fat diet on ghrelin hormone, glucose level, the liver enzymes AST and ALT.
**Materials and methods**: The experimental study was conducted using 24 healthy young male albino rat weighing 95±5 gram and age 2 month, one-way (ANOVA) were employed to determine a significance of differences.
**Results**: A significant increase $p \leq 0.05$ in glucose level of non-fasting control group compere with non-fasting low fat diet group, significant increase $p \leq 0.05$ in glucose level of control group fasting for 20h compared with low fat diet fasting for 20h group, significant decrease $p \leq 0.05$ when compares non-fasting low fat diet compares to 20h fasting low fat diet and significant decrease $p \leq 0.05$ when compares non-fasting control compares to 20h fasting control, while the effect of fasting and low fat diet on ghrelin hormone. A significant decrease $p \leq 0.05$ in ghrelin hormone level of non-fasting control group compere with non-fasting low fat diet group, significant increase $p \leq 0.05$ in ghrelin hormone of control group fasting for 20h compared with low fat diet fasting for 20h group, non-fasting control compares to 20h fasting control show a significant ($p \leq 0.05$) increase, Fasting with low fat diet cause a significant decrease $p \leq 0.05$ in ALT level, also in AST level there was a significant decrease $p \leq 0.05$ after 20h fasting.
**Conclusions**: The fasting and low fat diet have effected on ghrelin hormone, glucose level and fasting with low fat diet cause decrease in ALT level, also in AST level decrease after 20h fasting in male albino rats.

**KEY WORDS:** Fasting fat diet, low fat diet, Ghrelin hormone, Glucose level, liver function

## INTRODUCTION

Fasting is not a new concept; it has been practiced for hundreds of years and is recommended by a variety of religions for purification and cleansing purposes. Is fasting, nevertheless, beneficial to our physiology? Is there a chance that specific conditions will be harmed? Science nowadays is no longer based on empirical observations of fascinating things. Modern scientific activity demands that each given question be evaluated under well controlled and highly reproducible settings. Animal models, particularly rats, are crucial in this process, and a growing body of research suggests that fasting can improve human physiology and improve symptoms and quality of life in a variety of conditions. Obesity is one of the most evident, but there are also neurodegenerative illnesses, cancer, diabetes, cardiovascular disease, and sleep problems on the list [1]. Intermittent fasting (IF) is a kind of eating that alternates between fasting and eating intervals. It doesn't tell you which meals to consume, but rather when you should eat them. In this way, it's more correctly defined as an eating habit than a diet in the traditional sense [2]. Nutrients are substances that an organism needs in order to survive, develop, and reproduce. Carbohydrates, dietary fiber, fats, proteins, minerals, vitamins, and water are the seven primary types

of important nutrients for animals (including humans). Nutrients can be grouped as either macronutrients (carbohydrates, dietary fiber, fats, proteins, and water require in gram quantities) or micronutrients (vitamins and minerals needed in milligram or microgram quantities) [3]. Ghrelin is a hormone generated mostly by the stomach and released in lesser amounts by the small intestine, pancreas, and brain. Ghrelin serves a variety of purposes. Because it stimulates appetite, increases food intake, and promotes fat accumulation, it is known as the "hunger hormone." When given to people, ghrelin increases food intake by up to 30%; it circulates in the circulation and effects on the hypothalamus, a brain region important for hunger regulation [4]. Ghrelin also stimulates the pituitary gland to release growth hormone, which, unlike ghrelin, breaks down adipose tissue and encourages muscular development [5]. Alanine transaminase, or ALT, is an enzyme found mostly in the liver. ALT is released into the circulation when liver cells are destroyed. The quantity of ALT in the blood is measured by an ALT test. High levels of ALT in the blood can suggest a liver problem even if you don't have any symptoms of liver illness, such as jaundice (yellowing of the skin and eyes). Early diagnosis of liver illness may be aided by an ALT blood test. SGPT (serum glutamic-pyruvic transaminase) was

**Table I.** Component of low fat diet

| Low fat diet | | | |
|---|---|---|---|
| Nutritionalingredient | (100 kg) | metabolizable energy | crud protein |
| yellow corn | 51.5 | 1725 | 4.3 |
| Soybean meal | 35 | 780 | 16.8 |
| bran | 10 | 160 | 1.6 |
| Premix | 2.5 | 147 | 0.83 |
| corn oil | -------------- | | |
| limestone | 0.3 | | |
| Di-Calcium Phosphate | 0.7 | | |
| Total | 100 | | |
| chemical analysis of low fat diet | | | |
| metabolizable energy | 2812 | | |
| crud protein | 23.5 | | |
| total fat | 2.2 | | |
| The ratio of fat energy to total food energy | 7% | | |

**Table II.** Component of control diet

| Control diet | | | |
|---|---|---|---|
| Nutritionalingredient | (100 kg) | metabolizable energy | crud protein |
| yellow corn | 47 | 1575 | 4 |
| Soybean meal | 38 | 847 | 18.24 |
| Premix | 2.5 | 147 | 0.83 |
| corn oil | 11.5 | 1035 | ------ |
| limestone | 0.3 | | |
| Di-Calcium Phosphate | 0.7 | | |
| Total | 100 | | |
| chemical analysis of control diet | | | |
| metabolizable energy | 3604 | | |
| crud protein | 23.07 | | |
| total fat | 13.7 | | |
| The ratio of fat energy to total food energy | 34.2% | | |

the previous name for ALT. The SGPT blood test was previously known as the ALT blood test [6]. The enzyme AST (aspartate aminotransferase) is mostly located in the liver, although it can also be found in the muscles. AST is released into the circulation when your liver is damaged. The quantity of AST in your blood is measured by an AST blood test. The test can assist your doctor in determining whether you have liver injury or illness. Hepatitis, cirrhosis, mononucleosis, and other liver disorders can all be indicated by high AST levels in the blood. Heart issues or pancreatitis can also be indicated by high AST values [7].

## THE AIM

It aims to study the effect of fasting and low fat diet on ghrelin hormone, glucose level, the liver enzymes AST and ALT.

## MATERIALS AND METHODS

### METHODOLOGY

The experimental study was conducted during the period from April 2021 to August 2021, and the experiment was conducted using using 24 healthy young male albino rat (Rattusnorvegicus) weighing (95±5) gram and age 2 month. The animals were integrated with wooden shelves, under natural light 12 hours and 12 hours in the dark. The animals were placed in cages at laboratory temperature (23-25°C). Food and water were introduced daily, and kept for a week before the acclimatization experiment began. Adult Male Rat divided into two groups as following:

1- Non-fasting group: Low fat diet group 6 animal and Control group 6 animal.

2- Fasting for 20 hours group: Low fat diet group 6 animal and Control group 6 animal.

**Table III.** Effect of fasting period and LFD on glucose and ghrelin hormone levels of albino male rats (Mean ± SE)

| Parameter<br>Group (n=6) | Glucose (mg/dl) | Ghrelin (ng/mL) |
|---|---|---|
| CON1 | 156 ± 1.140 A b | 0.8384 ± 0.026 B a |
| LFD1 | 215.8 ± 2.596 A a | 0.5520 ± 0.010 B b |
| CON2 | 126.4 ± 3.043 B b | 1.3042 ± 0.033 A b |
| LFD2 | 156.6 ± 3.695 B a | 1.5872 ± 0.030 A a |
| LSD | 9.169 | 0.0878 |

**Table IV.** effect of fasting period and LFD on AST leveland ALT levelof albino male rats (Mean ± SE)

| Parameters<br>Groups(n=6) | AST level (IU/L) | ALT level (IU/L) |
|---|---|---|
| CON1 | 309 ±1.048 A a | 142 ± 2.712 Aa |
| LFD1 | 303.2 ±0.916 A b | 140.2 ± 2.576 A a |
| CON2 | 302.2 ±1.319 B a | 146 ± 2.738 A a |
| LFD2 | 300.2 ±1.854 Aa | 117 ± 2.701 B b |
| LSD | 3.49 | 14.3 |

Measurements include estimation of Serum Glucose, Serum Ghrelin hormone, liverenzyme Serum Alanine aminotransferase (ALT) and the activity of theliverenzyme aspartate aminotransferase (AST) in the blood is measured, the component of low fat diet, is shown in table (I), while the component of control diet is shown in table (II) [8].

## RESULTS

The difference capital letters refer to significant change (p≤0.05) when comparing fasting period of study group. The difference in small letter refer to significant change (p≤0.05) when comparing the nutrition type of the study group.

CON1 = non-fasting control diet group, CON2=20 hours fasting control diet group.

LFD1= non-fasting low fat diet, LFD2=20h fasting low fat diet.

The result of study shows that Effect of fasting and low fat diet on glucose level of control non-fasting group compere to non-fasting low fat diet show a significant p≤0.05 increase, control group fasting for 20h compared with low fat diet fasting for 20h group displayed a significant increase p≤0.05. The result of non-fasting low fat diet compares to 20h fasting low fat diet show a significant p≤0.05 decrease, non-fasting control compares to 20h fasting control show a significant p≤0.05 decrease in glucose level. The result of study shows that Effect of fasting and low fat diet on on ghrelin hormone level of control non-fasting group compere to non-fasting low fat diet illustrated a significant decrease p≤0.05, control group fasting for 20h compared with low fat diet fasting for 20h group displayed a significant increase p≤0.05. The result of non-fasting low fat diet compares to 20h fasting low fat diet displayed a significant increase p≤0.05, non-fasting

control compares to 20h fasting control show a significant p≤0.05 increase in ghrelin hormone level.

The difference capital letters refer to significant change p≤0.05 when comparing fasting period of study group. The difference in small letter refer to significant change p≤0.05 when comparing the nutrition type of the study group.

CON1 = non-fasting control diet group, CON2=20 hours fasting control diet group.

LFD1= non-fasting low fat diet, LFD2=20h fasting low fat diet.

The results shows that Effect of fasting and low fat diet on AST level of control non-fasting group compared with low fat diet non-fasting group illustrated a significant decrease p≤0.05. The result of control group fasting for 20h compared with low fat diet fasting for 20h group displayed a non-significant change p≤0.05. The result of non-fasting low fat diet compares to 20h fasting low fat diet show a non-significant change p≤0.05. The result of non-fasting control compares to 20h fasting control show a significant p≤0.05 decrease in AST level. The results shows that Effect of fasting and low fat diet on ALTcontrol non-fasting group compared with low fat diet non-fasting group illustrated a non-significant change p≤0.05. The result of control group fasting for 20h compared with low fat diet fasting for 20h group displayed a significant decrease p≤0.05. The result of non-fasting low fat diet compares to 20h fasting low fat diet show a significant decrease p≤0.05. The result of non-fasting control compares to 20h fasting control show a non-significant p≤0.05 change on ALT level.

## DISCUSSION

Fasting cause increase metabolic rate that lead to increase glycolysis to produce energy. Since this diet contains a

natural percentage of fat, the body does not activate the hormone glucagon, and at the same time the body will depend on the percentage of glucose come from this diet to generate energy. The results demonstrate that fasting with LFD induces a considerable drop in glucose levels [9]. Since the percentage of fat in this diet is very low, it caused a decrease in the level of glucose in the blood, and the glucose was clearly reduced after a 20-hour fasting period, and this led to an increase in the breakdown of glucose in the blood [10], and the other cause associated to ghrelin hormone level, fasting raises ghrelin levels, which lowers blood glucose levels because ghrelin raises the amount of insulin-secreting beta cells and serum insulin levels, which promote glucose metabolism [11]. Fasting increased the level of the ghrelin hormone in the fasting group. Different circumstances, including as fasting and pathological states, alter ghrelin production and secretion. Ghrelin levels in the blood rise during fasting and fall during eating. The rise in ghrelin levels during fasting is noradrenergic-mediated, while the subsequent drop is mediated by an increase in glucose and insulin [12]. After giving a low-fat diet to rats and measuring liver enzymes, we found decrease in ALT in group fasting for 20-hour. these results agree with human study and with animal model study [13-14]. There was a small decrease in the liver enzyme AST in the control diet and low fat diet in the case of fasting 20 hours with the control diet, as fasting improves liver function and reduces the liver enzyme this agree with [15]. Measurements of AST and ALT activity are two of the most essential assays for detecting liver damage; ALT is more specific to the liver than AST. The drop in ALT serum levels in fasting rats might be due to a decrease in tissue-specific enzymes and other intracellular proteins being released as a result of oxidative stress during metabolism [16-17].

## CONCLUSIONS

Overall, this study strengthens the idea that fasting causes an increased level of ghrelin hormone that leads to a decreased level of glucose, Fasting with low fat diet cause decrease in ALT level, also in AST level decrease after 20h fasting

## RECOMMENDATION

- The more penefet fasting priod is 20 houer.
- A low-fat diet is considered beneficial for health, because it lowers the level of sugar in the blood and increases the level of the hormone ghrelin.
- Use the same proportions of the diet, but with different ingredients such as casein and starch
- Ethical Clearance : Taken from University of Kufa ethical committee

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ORIGINAL ARTICLE

# LONG-TERM RESULTS OF TREATING PATIENTS WITH OPEN FRACTURES OF LOW-LEG BONES

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**ABSTRACT**
**The aim:** To analyse long-term results of treating patients with open fractures of low-leg bones within a specific time period and to describe concomitant problems.
**Materials and methods:** A retrospective study was carried out to evaluate the results of the final treatment of patients after one year and after five years. Patients were divided into two groups. Group I included 47 (61.84%) patients for whom the treatment method was changed from external fixation to internal fixation. Group II included 29 (38.16%) patients for whom the final treatment of an open fracture was performed with an external fixation device without changing the method. The study exclusion criteria were patients under 18 years old and patients with gunshot fractures of the lower leg bones.
**Results:** Patient treatment included initial surgical debridement and fracture stabilization with external fixation devices. In all patients, wound healing occurred within 30 days (on average, 24 ± 4.5 days). When evaluating the treatment of patients in group I, 32 (68.09%) of them underwent a one-stage removal of an external fixation device and osteosynthesis of fragments during the first 7-10 days. In 3 (20.0%) patients, osteosynthesis was performed with an intramedullary locking nail, in 12 (80.0%) patients – with a plate. The decision to change the fixation method was made taking into account clinical and laboratory parameters. It is noted that the treatment of patients with type III open fractures of the lower leg bones according to the Gustilo-Andersen classification, provided that the method of external fixation is replaced with an internal one, gives better results compared to osteosynthesis with external fixation devices.
**Conclusions:** Treatment of patients with high-energy injuries of the lower leg bones is a long-term process. It is possible to improve the therapy efficiency by changing the fixation method from external to internal one. In this regard, the main evaluative characteristics are such indicators as uncomplicated wound healing combined with the absence of laboratory and clinical signs of inflammation.

**KEY WORDS:** open fractures of lower leg bones, external fixation device, assessment techniques, scale, classification

## INTRODUCTION

Open fractures of the lower leg bones are often the result of high-energy events, including vehicle collisions (> 50%), a direct blunt blow to the tibia, sports injuries, or falls from a height [1, 2]. Over the past decades, scientific advances in wound care, prophylactic antibiotic therapy, fracture stabilization, and soft tissue reconstruction have made it possible to relieve and improve the condition of patients. However, the infectious marker is still a serious complication due to an open fracture of the lower leg bones. Treatment of these injuries requires a thorough and team approach. Therefore, the main principles of therapy are as follows: timely administration of antibiotics, careful surgical debridement of the wound, stable fixation of the fracture and replacement of soft tissue defects. Treating patients with high-energy injuries of the lower extremities is a long-term process and leads to a high percentage of unsatisfactory results. Therefore, these arguments serve as a convincing basis for studying this problem thoroughly and searching for the best ways to solve it [3].

## THE AIM

To analyzed the long-term results of treating patients with open fractures of the lower leg bones after one year and after five years and also assesse the problems faced by the patients during this time period.

## MATERIALS AND METHODS

During 2008-2016, 76 patients with open fractures of the lower leg bones which belong to the type III fractures according to the Gustilo-Andersen classification were treated at the Orthopedic and Traumatology Center of the Kyiv Region Clinical Hospital" (OTC KRCH) which is the municipal non-profit enterprise of the Kyiv Region Council. A retrospective study was carried out to evaluate the results of the final treatment of patients after one year and after five years. In the course of the analysis of results, 8 (10.53%) patients left the experiment after 5 years: communication with 5 patients was lost; 1 patient died (according to rela-

tives – from a cause not related to an open fracture), and 2 refused to take part in the study for personal reasons.

Patient treatment results were assessed using clinical and radiological methods. The following criteria were taken into account: X-ray image of the fracture union; subjective feelings of a patient; if there are any biomechanical disorders; efficiency restoration and if there are movement restrictions in adjacent joints, deformities and shortening.

The assessment was carried out using a modified Neer-Grantham-Shelton100-point scale of anatomical and functional results of treating fractures of long bones [4]. The motion range of the knee and ankle joint, as well as the functional status of the ankle joint were analyzed using the American Orthopedic Foot and Ankle Score (AOFAS) (scores from 0 to 100). The quality of life of patients was assessed using the 36-Item Short Form Survey (SF-36). Pain intensity, patient satisfaction with the effect of surgical treatment and therapy in general, daily work and sports activity were assessed using a 10-point VAS scale. The remaining variables are expressed as M ± SD, and categorical variables are expressed in percent. The Kolmogorov-Smirnov test was used to assess the normal distribution. Student's t-test was used for parametric variables when comparing groups. The Mann–Whitney U test was used for nonparametric variables. Fisher's test was used for the comparison of groups in terms of quality indicators. The correlation was assessed with Pearson's method. Differences were considered statistically significant if the probability of events was more than 95% in the general population and the risk of error did not exceed 5% ($p < 0.05$).

Among all patients who took part in the study, type IIIA injuries were observed in 41 (53.95%), type IIIB – in 26 (34.21%), type IIIC – in 9 (11.84%). The exclusion criteria from the study were such factors as the patients who are under 18 years old and who had gunshot fractures of the lower leg bones. The average age of the patients was 39 ± 6.5 years at the time of injury. The total number of patients under observation included 59 (78%) men and 17 (22%) women. In 36 (47.37%) patients injuries were caused by a road traffic accident, in 17 (22.37%) patients – due to a fall from a height, in the remaining 23 (30.26%) patients – by a home accident.

All patients were divided into two groups during treatment. Group I included 47 (61.84%) patients for whom the fixation method was changed from the external fixation to the internal one (with an intramedullary nail or a plate). Patients for whom the final treatment of an open fracture was carried out in an external fixation device (EFD) of the AO type or using the Ilizarov apparatus without changing the fixation method were included in group II, which consisted of 29 (38.16%) patients.

## RESULTS

The treatment approach for all patients included initial surgical debridement (ISD) and fracture stabilization with external fixation devices. The medical staff conducted a second examination within 24-72 hours, if necessary. In all patients, wound healing occurred within 30 days (on average 24 ± 4.5 days). At the first stage, skin defects were closed applying temporary methods: in 7 patients – with an artificial skin; in 24 patients – with a VAC system; further, autodermoplasty with a split flap was used in 14 cases, skin-fascial flaps were used in 19 cases, and free microsurgical flaps were used in 3 cases.

When evaluating the treatment of patients in group I, 32 (68.09%) of them underwent a one-stage removal of an external fixation device and osteosynthesis of fragments during the first 7-10 days: with an intramedullary nail in 9 (28.13%) patients and a plate in 23 (71.87%) patients. In 3 (20.0%) patients, osteosynthesis was performed with an intramedullary locking nail, in 12 (80.0%) patients – with a plate. The decision to change the fixation method was made taking into account two parameters:
1) clinical (primary wound healing, no necrosis and signs of inflammation);
2) laboratory (WBCs, ESR, CRP, and IL-6).
The dynamics of laboratory blood parameters were studied on the first day of injury, before and after the repeated examination, as well as before changing the fixation method.

In the course of treatment of patients of group I, specific complications were observed. In 4 (8.51%) patients, there was early suppuration in the fracture area after osteosynthesis with a plate. To stabilize the position, surgical interventions were performed to eliminate the focus of infection and to preserve the internal fixation device if possible. As a result of treatment, 3 (6.38%) patients showed elimination of the source of inflammation and subsequent fracture consolidation; one (2.13%) patient was diagnosed with chronic osteomyelitis. The therapy of chronic infection was conducted along with the fracture treatment with the external fixation device. In addition, repeated surgical interventions were performed to eliminate the source of infection, and repeated necrsequestrectomies were performed. The soft tissue defect was treated with the VAC system. Antibiotic therapy was prescribed taking into account intraoperative inoculation.

As a result of the treatment, it was possible to stop the infection and to heal the tibia. As a result, a pseudarthrosis of the tibia was formed in 5 (10.64%) patients: in 2 patients – after osteosynthesis with a plate; in 3 patients – after osteosynthesis with an intramedullary nail.

All patients underwent repeated surgical interventions to heal the fracture which made it possible to observe the consolidation of the fracture on X-ray images within 8-24 months.

After 1 year, final indicators were assessed in group I according to a modified Neer-Grantham-Shelton 100-point scale of anatomical and functional results of treating long bone fractures. Good results were observed in 27 (57.45%) patients, satisfactory – in 14 (29.79%) patients, unsatisfactory – in 6 (12.77%) patients. The average shortening of the affected limb was 1.42 cm; average extension and flexion in the knee joint were 167.5 degrees and 95 degrees, respectively; average dorsal extension and plantar flexion of the ankle were fixed at 110 degrees and 80 degrees,

respectively. The average score according to the AOFAS was 86.5 ± 5.9 points.

After 5 years, 6 patients discontinued participation in the study, so the results were assessed for 41 people. According to the Neer-Grantham-Shelton scale, good dynamics was observed in 30 (73.17%) patients, satisfactory – in 9 (21.95%) patients, unsatisfactory – in 2 (4.88%) patients. The average shortening of the affected limb was 1.42 cm; the average extension and flexion in the knee joint were 177.5 degrees and 60 degrees, respectively; average dorsal extension and plantar flexion in the ankle joint were 122 degrees and 75 degrees, respectively. The average score according to the AOFAS was 92.5 ± 5.3 points.

Evaluation of the survey data according to the HRQoL scale showed statistically significantly reduced results of the quality of life in age groups. The average pain intensity according to the visual analogue scale (VAS) was 2.8; the average score of patient satisfaction with the surgical effect was 9.2 points; overall satisfaction with treatment was 8.6 points; the average score of deterioration in daily and sports activity was 4.0 and 4.8 points, respectively.

The final treatment of patients in group II was carried out in an external fixation device: 25 (86.21%) patients – in rod devices, 4 (13.79%) patients – in an Ilizarov apparatus. In 3 (10.34%) patients of this group who had open fractures of type IIIC, amputation of the lower limb at the lower third of the thigh was performed. Acute suppuration was registered in 6 (20.69%) patients, which was followed by the development of chronic osteomyelitis (noted in one (3.45%) patient). Its treatment included repeated surgical interventions in order to eliminate inflammation in the bone tissue, as well as antibiotic therapy taking into account seeding tissue samples taken during the operation. The effect of the measures taken was observed when the fracture was fused and there was no inflammatory process in the projection of the lower leg bones.

In 7 (24.14%) patients, nonunion of the lower leg bones was observed. During the treatment of 4 (66.67%) patients, we achieved the complete union of the fragments and elimination of the inflammatory process; 2 (33.33%) patients were diagnosed with chronic osteomyelitis, which was confirmed radiographically and clinically. These patients, like the previous ones, who had an infection, underwent surgical treatment to remove the foci of the infectious process, fracture treatment with an external fixation device, antibiotic therapy and treatment of soft tissue defects using VAC systems. Elimination of chronic osteomyelitis and registration of complete fusion were observed within 1 year and 1.5 years after the start of therapy.

In group II, the final indicators were assessed after 1 year according to a modified Neer-Grantham-Shelton100-point scale of anatomical and functional results of treating fractures of long bones. Thus, good dynamics was observed in 9 (31.03%) patients, satisfactory – in 8 (27.59%) patients, unsatisfactory – in 12 (41.38%) patients (p = 0.012 in comparison with group I). The average shortening of the

affected limb was 1.67 cm; average extension and flexion in the knee joint were 172.3 degrees and 100 degrees, respectively; average dorsal extension and plantar flexion of the ankle were fixed at 110 degrees and 80 degrees, respectively. The average score according to the AOFAS was 79.8 ± 6.5 points.

After 5 years, the results were assessed in 27 patients, 2 of them refused to take part in further research. As a result, good results according to the Neer-Grantham-Shelton scale were noted in 8 (29.63%) patients, satisfactory – in 9 (33.33%) patients, unsatisfactory scores were noted in 10 (37.04%) patients (p <0.0001 in comparison with group I). The average shortening of the affected limb was 1.67 cm; average extension and flexion in the knee joint were 175.3 degrees and 70 degrees, respectively; average dorsal extension and plantar flexion of the ankle were at 100 degrees and 85 degrees, respectively. The average score according to the AOFAS was 82.1 ± 5.9 points.

The evaluated results of the questionnaire survey according to the HRQoL scale in age groups also showed statistically significant underrating of the quality of life. The average pain intensity according to the visual analogue scale (VAS) was 2.6; the average score of patient satisfaction with the surgical effect was 8.5 points; overall satisfaction with treatment was 8.4 points. The average score of deterioration in daily and sports activity was 3.8 and 4.2 points, respectively.

When analyzing the long-term results of treating type III open fractures of the lower leg bones according to the Gustilo-Andersen classification, the number of good indicators in group I increased by 15.72%, and in group II – by 2.3%. The number of unsatisfactory results in group I decreased by 7.89%, and in group II – by 4.34% (p < 0.01).

A significant inverse correlation (p < 0.01) was observed between the intensity of pain and all subgroups of the SF-36 (r = -0,428 – -0,757), AOFAS, VAS scores, especially in terms of life satisfaction, deterioration in daily, work and sports activity. Additionally, there was a positive correlation between the level of dorsal extension in the ankle joint and subgroups of the SF-36 general health scale (r = 0.358; p = 0.035). The SF-36 also shows an inverse correlation between total treatment time and patient satisfaction with the result (r = 0,358; p = 0,003), limb functioning (r = -0,344; p = 0,04), mental health problems (r = -0,339; p = 0,032).

Thus, treating patients with type III open fractures of the lower leg bones according to the Gustilo-Andersen classification by changing the fixation method from external fixation to internal one leads to significantly better results than the use of osteosynthesis with external fixation devices.

## DISCUSSION

Today, the treatment of patients with open fractures of the lower leg bones is a relevant issue for an orthopedic traumatologist. In their practice, fractures of the lower leg bones are common – from 23 to 35.5% of all injuries

of the musculoskeletal system. The authors would like to emphasize that open injuries of the lower leg bones usually dominate (65.3-77.8%) among open injuries of the bones of the skeleton [5].

Currently, the main feature in treating open fractures of the lower leg bones is a relatively high percentage of cases of non-union – 25% cases of the total number of unsatisfactory treatment outcomes. At the same time, the proportion of patients with fractures and post-traumatic defects of the leg bones complicated by osteomyelitis reaches 30%.

These complications remain relevant for several decades, which also indicates the high importance of studying the issue associated with open fractures of the lower leg bones in the current conditions. The reasons for the unsatisfactory results of treating patients with open injuries of the lower leg bones are: lack of consensus among traumatologists regarding the choice of optimal treatment approach for patients, taking into account the severity of the open injury; insufficiently thorough primary surgical treatment of wounds; limited use of drainage and soft tissue plastic closure techniques [6].

When treating all patients with open fractures, we adhered to a consistent approach with regard to this matter. At the initial stage (when the patient was admitted to us), initial surgical debridement and stabilization of a fracture with an external fixation device were performed in order to create optimal conditions for wound healing and fracture union.

Initial surgical debridement includes radical debridement of a wound, intraoperative irrigation of a wound and its closure. This set of measures is always used to prevent infection after severe trauma to a limb [7].

Moreover, we believe that additional surgical treatment plays an important role in the further assessment of tissue viability. On day 2-4 our patients (83% of the total number of patients) were re-examined to assess the quality of initial surgical debridement, which was carried out primarily in other hospitals. We actively used the VAC system (in 86% of cases) for temporary wound closure. This method promotes effective alignment of the wound edges, reduces edema, forms granulation tissue and improves blood supply, as well as ensures the evacuation of separated and infected wound material. Such measures ensure rapid wound healing and subsequently allow the use of flaps for the final closure of soft tissue defects. It should be noted that the final closure of wounds is recommended no later than in 3 weeks.

Stable fixation of the fracture is a necessary step in treating patients with open fractures of the lower leg bones. In this regard, the method of controlled transosseous osteosynthesis, the founder of which is Academician G.A. Ilizarov, is the "gold standard". This method is indispensable as a means of temporary fixation of fractures of the lower leg long bones in patients with polytrauma and with the development of compartment syndrome [8].

Some authors consider the use of an external fixation device as the most biologically justified method of skeletal stabilization which helps to maintain blood supply to the soft tissues of the lower leg [9]. The advantages of this method are the relative speed and ease of use, access to soft tissues during their further reconstruction.

It should be emphasized that when an external fixation device is used for a long period there are often inflammation of the soft tissues around the pins and nails, neurotrophic disorders in the lower leg and foot, lymphostasis, and persistent contractures of nearby joints develop. The external fixation devices require constant medical supervision and patient care. In addition, their use significantly reduces the patient's quality of life. Therefore, many authors consider the optimal solution to replace the external fixation device with the internal one after wound healing to adapt the fragments, as well as to improve the functional and social rehabilitation of patients [7, 9].

In this regard, the OTC KRCH successfully used a two-stage approach of operative fixation of bone fragments in case of lower leg open injuries. To improve the results of treatment, we removed the external fixation device and changed the osteosynthesis method in 61.84% of patients. The operation to change the method was carried out only if: the patients did not have deep purulent complications and showed normalization of body temperature; there were no laboratory signs of inflammation (WBCs, ESR, CRP, and IL-6).

Changing the fixation method from the external to the internal one made it possible to adapt the fragments, perform bone grafting, and ensure stable osteosynthesis of the fracture.

When evaluating the results of treating patients with open fractures of the lower leg bones within 1 year and 5 years after the injury, we took into account complications that arose in the late period. The evaluation of the treatment method's effectiveness was based on clinical data, X-ray images, as well as information on the degree of impairment of the static and dynamic functions of the limb. The following signs were noted in patients for whom the fixation method was changed from external to internal after 1 year and after 5 years:

1) reducing the risk of developing pyoinflammatory complications by 65% ($OR_{I/II} = 0.35$ (95% CI: 0.07-1.7; $p = 0.127$));
2) there is no rotational instability of bone fragments;
3) there is no delayed consolidation and development of false joints;
4) the physiological axis of the injured limb is restored;
5) it is possible to refuse to use an external fixation device.

When analyzing the intensity of the pain syndrome after 5 years according to the VAS scale in both groups, patients note relatively minor pain and relative satisfaction with surgical and general treatment. Despite this fact, professional and sports activity was significantly weaker throughout the subsequent life of most patients. Every aspect of their quality of life was underrated according to the 36-Item Short Form Survey (SF-36) – physical limitations and psychological problems became the main reasons that led to this fact. The overall duration of treatment correlates

with low patient satisfaction and an increase in the level of physical limitation, which leads to further problems, including psychological ones. Such an indicator as the intensity of pain became the main factor that negatively correlated with the patients' quality of life.

Patients of both groups (more in group II) showed slight limitations in the range of motion in the knee and ankle joints. Limited dorsal extension of the ankle joint negatively correlates with the general health condition, as shown in the SF-36. Low scores according to the American Orthopedic Foot and Ankle Score (AOFAS) indicate limited motion in the ankle joint. Additionally, the AOFAS correlates with patient satisfaction with the result, deterioration in their daily life, including professional and sports activity. This emphasizes the importance of restoring the ankle joint for the person's daily active participation in social activities.

Moreover, the limitation of foot extension is further correlated with the limitation of activity in daily life and general health, as seen in the SF-36. These results prove that bone union in combination with successful soft tissue closure of the nonunion site should be the primary goal of treatment in such patients, despite the duration of therapy and the additional number of surgical interventions. Moreover, the limitation of movement in the ankle joint, which may arise as a possible result of prolonged immobilization or the formation of scar tissue, should be taken into account during the rehabilitation period.

In our patients, the average amplitude of extension in the ankle joint was > 75 degrees in group I and > 80 degrees in group II after five years. At the same time, there is a significant limitation of movement in everyday life, for example, when walking on an uneven surface and going up/downstairs.

## CONCLUSIONS

1. Treating patients with high-energy injuries of the lower leg bones is a long-term process and leads to a high percentage of unsatisfactory results. In this regard, it is important to apply an integrated approach, minimize risks to the patient's health and study the verified opinion of specialists, taking into account all factors to achieve an optimal therapeutic and surgical effect.

2. For the successful treatment of open fractures of the lower leg bones, an adequate initial assessment of the injury and readiness for multistage processes regarding the implementation of a whole complex of procedures are required: initial surgical debridement, fracture stabilization with an external fixation device, and, if necessary, the use of "second look" and timely closure of skin defects. Changing the fixation method from the external to the internal one will help improve patient treatment results. The main evaluative characteristics are such indicators as uncomplicated wound healing combined with the absence of laboratory and clinical signs of inflammation.

3. Despite the long period of rehabilitation of patients, we did not notice a significant improvement in the quality of their life. According to the evaluated SF-36, AOFAS, VAS scores, the patients experienced the greatest discomfort due to pain, decreased limb functioning, inability to return to their previous work and usual lifestyle. These observations were carried out in a group of patients with significant soft tissue trauma, for whom it was impossible to change the treatment method by replacing the external fixation with the internal one.

4. The urgent need to continue research in this field and find rational solutions is due to the factors described in this article. The problem of patients diagnosed with an open fracture of the lower leg bones has not been resolved yet and is far from obtaining more favorable outcomes.

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ORIGINAL ARTICLE

# STRESS LEVELS REGARDING COVID-19 PANDEMIC AMONG NURSING STUDENTS AT UNIVERSITY OF SULAIMANI, KURDISTAN REGION, IRAQ

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**ABSTRACT**
**The aim:** The study aimed to assess prevalence levels of stress among undergraduate college of nursing students at university of Sulaimani, Kurdistan Region/Iraq.
**Materials and methods:** A total of 60 nursing students were selected from college of nursing Sulaimani University by convenience sampling used in their cross-sectional study. The perceived stress scale (PSS-10), and socio-demographic characteristics included in the self-reported questionnaire. The period of data collection started from October 25th, 2021 to November 25th, 2021. The data analysis performed by using SPSS version 25. Ethical approval was considered.
**Results:** The results of this study showed that the overall prevalence rate of stress was determined as 53.3 % of students at moderate level which was more prevalent among fresh man students within age 18-19 years old 93.4 %, while low level of stress observed among senior students within age 20-21 year old (25%) (p<0.01). The other socio-demographic factors were not associated with levels of stress. The finding indicated that 40% of the students were infected with covid-19 in last period while the pandemic is still ongoing.
**Conclusions:** nursing students were experienced moderate to low level of stress regarding covid-19 pandemic regardless of socio-demographic characteristic, only age factor significantly associated with the variance of stress.

   **KEY WORDS:** Stress, covid-19, nursing students

## INTRODUCTION

The covid-19 pandemic has separated across the world [1], and has had a key impact on people lives [2]. The covid-19 this disease represents a unique global challenge because of its contagiousness and the severity of respiratory infections it can causes, sometime lethal [3] along with it a considerable degree of psychological distress, specially, not only the danger of contagion but also various changes in habits due measures of self-isolation and social distancing [4-5]. The distress occurs when person's ability to cope with stressors is insufficiently in knowledge regarding control and inadequate treatment measures which effecting people's daily lives physically and psychologically and such challenges can rise stress [6]. Stress viewed as a condition in which the human system responds to changes in its normal balance as result from changes in the environments that is perceived as challenge, threat or danger [7]. The transactional theory of stress assumes that stress is a relationship between the person and the environment that is appraised by the person as taxing or exceeding his/her resources and end angering their wellbeing [8]. Moreover, a cognitive evaluation of the significance of the event or occurrence, the event precipitates response, on the part of the individual and the responses is influenced by one's perception of the event and the appraisal positive or negative transition and changes can be sources of stress [7-8]. Stress has different types that might people experience which includes acute

stress, chronic stress, episodic acute stress and eustress [9]. Stress often play an important role in precipitating physical and mental health problems in those who are predisposed, thus awareness of stressors and interventions helpful for suffering people to cope with stress itself [10]. These awareness and understanding the covid-19 disease in the context of mental health is the responsibility of the mental health professionals towards people. However Zhang [11] reported that the prevalence of depression, anxiety and stress related symptoms being 50.7%, 44.7% and 73.4% respectively, among medical team during out break period of covid-19 pandemic. College of nursing students experienced stress, intermittently in difficult times mainly due to perceived stress in turn as it is associated with virus transmission practically during practical training in various field of nursing in hospitals and considered as front line contact with patients in the line with presented evidence and to provide further richness to existing frame works of covid-19 disease on protective for mental health and assessment is the first step in mental health management, thus present study concern to assessing the levels of stress and the role of variables in contributing college of nursing student's stress at Sulaimani University in Kurdistan region of Iraq. To the best of author knowledge no previous study has investigated the prevalence levels of stress among such population that is nursing undergraduate students in Sulaimani city in Kurdistan Region of Iraq. Therefore

this study is important to identify vulnerability of student to stress, and protective measure could be taken, hence to limit negative psychological outcome among such group of students.

## THE AIM

The study aimed to assess prevalence levels of stress among undergraduate college of nursing students at university of Sulaimani, Kurdistan Region/Iraq.

## MATERIALS AND METHODS

### STUDY DESIGN AND SETTING

Quantitative, cross-sectional design was carried out in college of nursing at Sulaimani University/Ministry of Higher Education and Scientific Research in Kurdistan Region/Iraq. The period of data collection started from October 25th, 2021 till November 25th, 2021.

### SAMPLE SIZE ESTIMATION

The G-power correlation model was used for estimating the sample size with a 95% confidence interval (CI) with medium effect of 0.5-0.8 power and level of significance p-value of 0.05. The total sample was calculated to be 60 undergraduate students in college of nursing.

### THE STUDY SAMPLE

Non-probability, convenience sampling technique was used to select 60 students were recruited from the college of nursing. The inclusion criteria for sample selection was the students agree to participate in this study, of both genders and about 15 students chosen in year first, second, third and fourth stage. The students who not interested to participate in this study were excluded.

### THE STUDY INSTRUMENT

A self-reported questionnaire was constructed as an instrumental tool for data collection. it consisted of three parts. The first part includes student's socio demographic characteristics. The second part related to the student's infected or not of covid-19 disease. The third part was the perceived stress scale (pss-10). It is 10-items self-report standardized instruments measuring the degree to which situation in one's life are appraised as stressful. Each item scored on a 5-point Likert scale (never=0, almost never=1, sometime=2, fairly often=3, very often=4). The total score is obtained by summing all items and the higher the score the grater the perceived stress. The score are obtained by reversing responses to the items 4, 5, 7 and 8, the scoring ranging from 0-60. The guidelines scores ranging were from 0-19 considered low stress, 20-39 considered moderate, and the score from 40-60 considered high perceived stress. The questionnaire was translated to Kurdish language through forward-backward procedure. The face validity of the questionnaire was determined through a panel of five experts, and reliability was determined by the computation of the interclass coefficient correlation by application of statistical package for social science program (SPSS-version 25), the result was 0.91 such estimation means the questionnaire be reliable.

## PROCEDURE OF DATA COLLECTION

The administration of the questionnaire takes between 25 to 30 mints to complete. The self-reported technique of data collection includes close-ended items. The students asked to answer each item by writing on the questionnaire without mention personal name.

## STATISTICAL ANALYSIS

The statistical data analyses were performed by using statistical package for social science software version 25. Descriptive statistics includes frequency and percentage and the inferential statistics includes chi-square $\chi^2$, fisher exact test are used to determine the significant relationship of socio demographic data with the level of stress among sample.

## RESULTS

A total of 60 undergraduate nursing students participate in this study. Table I showed the student's socio demographic characteristics.

The result in this table revealed that the student's age ranged from 18 to 26 years, the majority of them was single (91.7%) with sufficient economic status (63.3%) and about two third of them 60% living in dormitory during data collection. The result of the present, table (II) indicated that more than one third of the students (40%) infected with covid-19 during the past year.

According to the severity of stress among the students, the results in table II showed that slightly more than half of student (53.3%) had a moderate level of stress, 45% low levels, and very little proportion of the students (1.7%) had sever level of stress. Table III showed the association between socio demographic variables with severity levels of stress, the results revealed that there are no statistical significant relationship (p>0.05) between levels of stress with genders (p=0.336), marital status (p=0.887), economic status (p=0.347), residency areas (p=0.957), infection of covid-19 (p=0.769), while the age factor had a significant relationship with levels of stress (p>0.01).

Moreover, table IV indicated that moderate level of stress was more prevalent among nursing students 23.4% within age group 18-19 years compared to 11.7% of students within age 20-21 years, 18.4% of them within age group 22-23 years and 0% of students in age group 24-25 years, furthermore the result of this table showed that low level of stress was more prevalent among nursing students within age 20-21 years compared to other groups of students (Fisher exact test 0.025, p-value 0.01).

**Table I.** student's socio-demographic characteristics

|  | Characteristics | F | % |
|---|---|---|---|
| Age | 18-19 years | 18 | 30 |
|  | 20-21 years | 23 | 38.3 |
|  | 22-23 years | 17 | 28.2 |
|  | 24-25 years | 2 | 3.4 |
| Gender | Female | 30 | %50 |
|  | Male | 30 | %50 |
| Marital Married | Married | 5 | 8.3 |
|  | Single | 55 | 91.7 |
| Economic status | Sufficient | 38 | 63.3 |
|  | Barely-sufficient | 21 | 35.0 |
|  | Insufficient | 1 | 1.7 |
| Residential area | Urban area | 31 | 51.7 |
|  | Suburban | 26 | 43.3 |
|  | Rural | 3 | 5.0 |
| Current residency | Current home | 24 | 40 |
|  | Dormitory | 36 | 60 |
| Infected with covid-19 | Yes | 24 | 40 |
|  | No | 36 | 60 |
|  | Total | 60 | 100.0 |

**Table II.** Distribution of the stress according to stress levels

| Stress level | F | % |
|---|---|---|
| Low | 27 | 45.0 |
| Moderate | 32 | 53.3 |
| Sever | 1 | 1.7 |
| Total | 60 | 100.0 |

**Table III.** Association between socio demographic characteristics with stress level of students

| Variables | Fisher exact test | p-value | Sig |
|---|---|---|---|
| Age | 0.025 | 0.01 | S |
| Gender | 0.195 | 0.336 | NS |
| Marital status | 1.000 | 0.883 | NS |
| economic status | 0.797 | 0.347 | NS |
| Residence | 1.000 | 0.957 | NS |
| Stage year | 0.663 | 0.520 | NS |
| Infected with covid-19 pandemic | 0.343 | 0.764 | NS |

S= significant
NS= non-significant

## DISCUSSION

The result of this study showed that the overall prevalence rate of stress levels was determined as 53.3% at moderate level in most general sense, stress is a condition that results when person-environment transaction lead the person to perceive a discrepancy, whether read or not, between the demands of situations and biological, psychological or social resources of the person [12] moderate level often encounter in something new or exciting, dangerous and hazard situations and sometimes lead to mental health problems of don't manage. This finding was similar to other previous studies [13-15] who reported that health professionals including nurses may be more impacted consequences of the covid-19 pandemic including psychological and physiological responses such as anxiety, stress, depression, and fear particularly among staff that were on the front line. This result on the line of study carried out by [16] showed that health professionals (n=906) who participated in a survey head moderate to severe depression and moderate to extremely severe stress. Moreover the result of this study indicated that 40% of the samples were infected by covid-19 disease during last period. This finding suggested that nursing students are also most vulnerable population group of covid-19 infection, and thus presence of stress may be attributed of being infected or transferred to their family members and other people. Machado et al supported the result of this study and pointed that in previous meta-analysis study showed that forty-three articles were reported the presence of symptoms or mental problems the covid-19 pandemic, especially the most vulnerable populations group in elderly and health professionals [1]. Furthermore, the results of the current study revealed that moderate level of stress was significantly more prevalent among students within age group 18-19 years (28.4%, p> 0.01) as this group of students represented freshman students while low level of stress more prevalent among students with in age 20-21 years (25%) which may be represents scientific nursing students, as compared to other age groups (table IV). This finding was in agreement with some like

**Table IV.** Association between age groups with stress level

| Age group | Stress levels | | | | | |
|---|---|---|---|---|---|---|
|  | Low | | Moderate | | Sever | |
|  | F | % | F | % | F | % |
| 18-19 | 4 | 6.7 | 14 | 23.4% | 0 | 0% |
| 20-21 | 15 | 25 | 7 | 11.7% | 1 | 1.7% |
| 22-23 | 6 | 10 | 11 | 18.3% | 0 | 0% |
| 24-25 | 2 | 3.3 | 0 | 0% | 0 | 0% |
| Total | 27 | 45% | 32 | 53.3% | 1.0 | 1.7% |

Fisher exact test = 0.025, p-value 0.01, significant

studies which reported that age consider independent variable associated with the variance levels of stress [17-18] furthermore, the result of this study showing that there was no significant association with stress levels with marital status, economic status, residential area, current residency and state of infection with covid-19 [19]. This result may be due to that covid-19 pandemic affected students regardless of most of their Sociodemographic characteristics, or the current finding may attributed to methodology and small sample size which influences the analysis of results; additionally their findings need further investigation. However, the present study build first step in management of covid-19 among nursing students while the pandemic is still ongoing.

## CONCLUSIONS

The results of the current study detected that almost all college of nursing students were experienced moderate to low levels of stress during covid-19 pandemic, regardless of most of their socio demographic status, only age factor significantly with the variance of stress. The moderate level of stress more prevalent in freshman students within age group 18-19 years while low level of stress more prevalent among senior students within age 20-21 years old. Moreover the results revealed that tow-third of the students current of living in dormitory, and slightly more than one-third of them were infected with the covid-19 disease in the last period; while the pandemic is still ongoing.

## RECOMMENDATION

This study recommended that mental health clinic should be established in the college for interventions and reduces psychological problems among students. And psycho education programs should be implemented to the nursing students to reduce stress and to change lifestyle, furthermore applying further protective measures including masks, gloves, avoiding crowded places particularly in dormitory and to be vaccinated against covid-19. The nursing students are in the front line and need to be psychological and social supported. Moreover encourage further epidemiological studies targeting mental health problem including stress among university students.

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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

ORIGINAL ARTICLE

# PROGNOSTIC CRITERIA OF EXCESSIVE BODY WEIGHT DEVELOPMENT AMONG SCHOOLCHILDREN BY THE RESULTS OF ANAMNESTIC SURVEY

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**ABSTRACT**

**The aim:** To elaborate prognostic criteria to assess the risk of excessive body weight development among schoolchildren on the basis of hereditary and behavioral predictors.

**Materials and methods:** 90 parents of school age children were interviewed by means of the social study method.

**Results:** Hereditary status (1 and 2 congeniality degree to type 2 diabetes mellitus, arterial hypertension, excessive body weight, cases of myocardial infarction and/or stroke available among relatives) and behavioral characteristics (peculiarities of diet and physical activity of a child) were studied among school age children. The risks promoting development of an excessive body weight under conditions of hereditary and behavioral factors were assessed. The prognostic matrix elaborated enables to predict development of an excessive body weight of a child under a comprehensive effect of unfavorable hereditary and behavioral characteristics with a high accuracy (AUC = 0,88, Std. Dev. = 0,0451).

**Conclusions:** The method elaborated enables to find children with the risk of an excessive body weight development and introduce individualized prophylaxis measures in order to prevent development of obesity and diseases associated with an excessive body weight.

**KEY WORDS:** children, excessive body weight, risk factors, prognostication

## INTRODUCTION

Nowadays assessment of factors, variable by their nature and origin, influencing upon health, and prognostication of development of diseases among population have become more significant within the system of public health care. Obesity in childhood has an irreversible effect on the human health, causes unfavorable medical, social and economic consequences [1-3]. Important measures in order to solve this issue include investigation of hereditary risk factors promoting development of an excessive body weight among children and unfavorable behavioral family characteristics, organization of their registration and prevention applying health-improving technologies [4]. In order to assess hereditary and unfavorable behavioral risk factors promoting development of an excessive body weight and obesity among children within the frame of introducing medical-social monitoring of obesity among schoolchildren, anamnestic survey of parents is elaborated and introduced into health care institutions included into the program of preventive examinations of children.

## THE AIM

The aim – elaborate prognostic criteria to assess the risk of excessive body weight development among schoolchildren on the basis of hereditary and behavioral predictors.

## MATERIALS AND METHODS

The study was conducted among 90 respondents, parents of children who were consulted by a district pediatrician with the purpose to undergo annual preventive medical examination of their children. Heredity, dietary anamnesis and peculiarities of physical activity of children were studied by means of the social study method – the survey of questionnaire – substantiated on the basis of the Clinical Practice Guideline developed by the European Society of Endocrinology «Pediatric Obesity – Assessment, Treatment and Prevention», Styne Dennis M. et al., [5] and the results of the WHO collaborative cross-national study «Health behaviour in school-aged children (HBSC) study», Inchley Jo [6]. The questionnaire was simple to use. It did not take much time to fill it in. The survey was conducted during preliminary consulting when parents wrote an informed consent concerning medical examination of their children.

The questionnaires obtained were sorted out into two groups: I group – 75 questionnaires from the parents whose children had an excessive body weight (body mass index within 85-97 percentiles for a corresponding age and sex) and obesity (body mass index more than 97 percentiles for a corresponding age and sex), II group – 15 questionnaires from the parents whose children had normal body weight (body mass index within 5-85 percentiles for a corresponding age and sex).

Statistical significance of value differences between the groups of comparison was determined by means of Fisher transformation $p_\varphi$ applying the computer program Statictica 10 StatSoft.
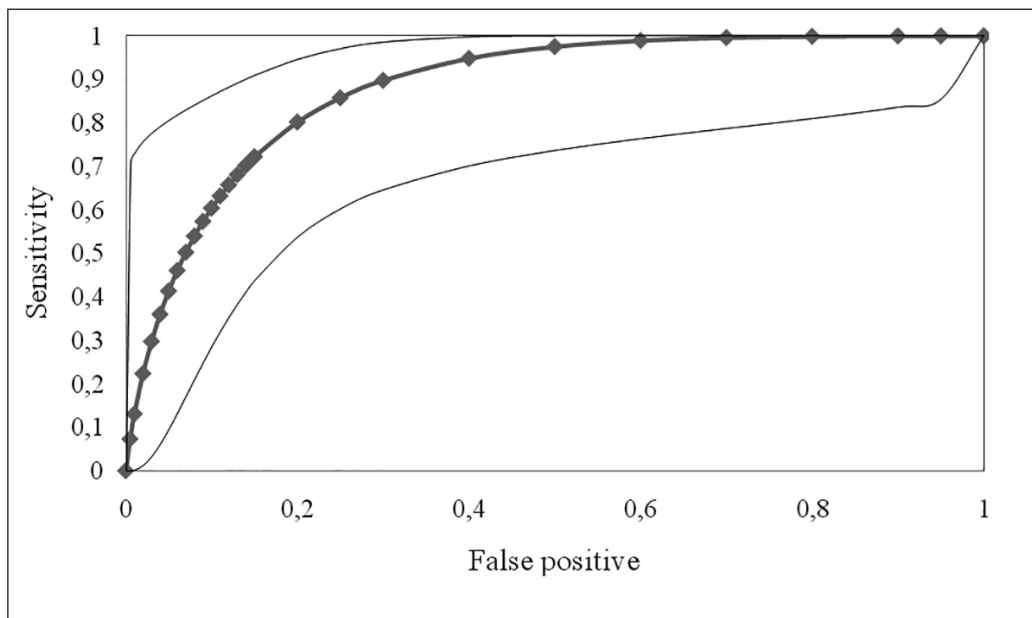
**Fig. 1.** ROC-curve to predict development of an excessive body weight among schoolchildren

**Table I.** Risk factors promoting development of an excessive body weight among schoolchildren

| Characteristics | Groups of observation | | $p_\varphi$ | OR; 95% CI |
| --- | --- | --- | --- | --- |
| | I, % | II, % | | |
| Compromised heredity with T2D | 46,7 | 13,3 | 0,002 | 6,7; 1,4–31,5 |
| Compromised heredity with AH | 37,3 | 13,3 | 0,017 | 4,6; 0,97–21,5 |
| Compromised heredity with MI/S | 26,7 | 13,3 | > 0,05 | 2,8; 0,6–13,2 |
| Relatives of 1 and 2 degree of relationship with EBW/O | 81,3 | 26,7 | < 0,001 | 15,3; 4,3–54,3 |
| Intake of chips, fast food, sweet drinks more than 3-4 times a week | 88,0 | 66,7 | 0,032 | 3,7; 1,02–13,2 |
| Intake of vegetable less than twice a week | 34,7 | 6,7 | 0,004 | 7,4; 1,0–59,7 |
| Intake of fruits less than twice a week | 26,7 | 6,7 | 0,024 | 5,1; 0,6–41,3 |
| Intake of meat products less than twice a week | 24,0 | 6,7 | 0,039 | 4,4; 0,5–34,0 |
| Intake of fish products less than twice a week | 56,0 | 26,7 | 0,016 | 3,5; 1,02–12,0 |
| Sitting at an electronic appliance over 3 hours every day | 33,3 | 13,3 | 0,045 | 3,25; 0,7–15,5 |
| Physical activity less than 0,5 hours a day | 86,7 | 60,0 | 0,014 | 4,3; 1,3–14,8 |

Confidence intervals for these values assumed as 95% were calculated with boundary risk of error less than 5% (p<0,05). Probability of development of an excessive body weight of a child under conditions of anamnestic aggravation and unfavorable behavioral characteristics was assessed according to the odds ratio indices [7].

Prognostic matrices were developed by Bayes' method by mans of normalizing intensive indicators and calculation of in-

tegrated indices. Normalized intensive indicator was calculated according to the formula: N = f/I, where f – sign frequency and/or characteristic frequency in the group of comparison (intensive indicator, %), F – normalization index (sign frequency and/or characteristic frequency according to the results of the study in both groups, %). Integrated index was calculated according to the formula: I = N*OR, N – normalized intensive indicator, OR – odds ratio. The prognostic table included the signs with

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**Table II.** Prognostic matrix for a comprehensive assessment of risk promoting development of an excessive body weight among schoolchildren

| Characteristics | Index for normalization | Normalized intensive indicator | | Integrated index | |
|---|---|---|---|---|---|
| | | I | II | min. | max. |
| Compromised heredity with T2D | 40,7 | 1,15 | 0,33 | 2,2 | 7,7 |
| Compromised heredity with AH | 33,0 | 1,13 | 0,4 | 1,8 | 5,2 |
| Relatives of 1 and 2 degree of relationship with EBW/O | 71,4 | 1,14 | 0,37 | 5,7 | 17,4 |
| Intake of chips, fast food, sweet drinks more than 3-4 times a week | 83,5 | 1,1 | 0,8 | 3,0 | 4,1 |
| Intake of vegetable less than twice a week | 29,7 | 1,2 | 0,2 | 1,5 | 8,9 |
| Intake of fish products less than twice a week | 50,5 | 1,1 | 0,5 | 1,8 | 3,9 |
| Physical activity less than 0,5 hours a day | 81,3 | 1,1 | 0,7 | 3,0 | 4,7 |
| Calculated index of risk: minimal and maximal | | | | 19 | 51,9 |

## RESULTS

significant odds ratio indices which were determined by 95% odds ratio values. Prognostic value of the effect of hereditary aggravation and unfavorable behavior promoting development of an excessive body weight in children was assessed by the AUC balanced accuracy with interpretation of the result obtained by the AUC expert interval scale (Winham et al., 2010, Swets, 1988).

## RESULTS

Investigation of hereditary characteristics demonstrated that 75 (83,3%) children had compromised anamnesis concerning development of an excessive body weight – relatives of 1 and 2 degree of relationship with type 2 diabetes mellitus (T2D), arterial hypertension (AH), an excessive body weight and/or obesity (EBW/O), cases of myocardial infarction and/or stroke (MI/S). At the moment of conducting the survey 15 children did not have compromised family anamnesis. The majority of them (66,7%) had normal body mass index for their age and sex. Occurrence of unfavorable hereditary and behavioral characteristics found concerning development of an excessive body weight and risks promoting it are presented in Table I.

Hereditary compromised myocardial infarction and/or stroke were excluded from a comprehensive assessment of risks promoting development of an excessive body weight of schoolchildren among biological characteristics essential for the construction of a prognostic matrix. Among behavioral factors we have chosen characteristics possessing a reliable probability and value concerning development of an excessive body weight of a child: excessive intake of chips, fast food, and sweet drinks, insufficient intake of fish and vegetables, low physical activity. The prognostic matrix including hereditary and behavioral risk factors promoting development of an excessive body weight and/or obesity among schoolchildren is presented in Table II.

The matrix includes all the biological and behavioral risk factors chosen for prognostication with the values of calculated indices, that is, indices to perform normalization, normalized intensive indicators and integrated indices. The range of the risk values by a complex of factors was determined according to the integral indices.

Thus, the value of a minimal risk promoting development of an excessive body weight of a pupil is the sum of minimal integrated indices: 2,2 + 1,8 + 5,7 + 3,0 + 1,5+ 1,8 + 3,0 = 19. The maximal value of the prognostic risk is 51,9. The range of risk (19–51,9) was determined on the basis of the prognostic matrix (19–51,9), which enabled to identify the interval values: low risk (19–29,9), average risk (30–40,9), high risk (41–51,9). The quality of the prognostic assessment was checked by means of ROC-curve (Fig 1.).

Sensitivity of risk assessment of an excessive body weight among schoolchildren under conditions of integrated index of a combined unfavorable effect of hereditary and behavioral risk factors more than 30,0 is 82,9%, specificity – 77,8%. The square under the curve 0,88 (Std. Dev. AUC = 0,0451) is indicative of a high quality of the prognostication method.

## DISCUSSION

Our results obtained coincide with the results of other modern researches which confirm that child's physical activity depends on a complicated relationship of the genetic background with family behavioral and dietary stereotypes, environmental factors, as well as exposition and intensity of a comprehensive unfavorable effect of these factors on the child's organism [8, 9].

According to our data hereditary susceptibility to the development of an excessive body weight of a child is of the greatest value among biological factors (odds ratio = 15,3; 95%CI: 4,3–54,3). The international study on pediatric obesity, the lifestyle and environment (Lifestyle and the Environment – ISCOLE) determined that in the families where parents have an excessive body weight a percentage of children with an excessive weight is 84,1%. The scientists recommend learning the family status while following the state of health and development of a child [10].

In the process of family socialization a child assimilates a certain lifestyle including an unfavorable one consisting of unhealthy diet and low physical activity, which results in the development of an excessive body weight [11, 12]. The intake of chips, fast food and sweet drinks more than 3-4 times a week were found to be the most common behavioral factors. The percentage of such children was 83,5%. Fast food is confirmed to be characterized by low dietary value, very high content of fats and saturated fatty acids, which excess in the diet results in

increasing the body weight. Sugar intake increases energy but decreases dietary value of food.

Physical activity increases energy loss. Excessive food and sedentary lifestyle promote development of an excessive body weight considerably. Sedentary life is associated with longer time passed away in front of electronic appliances (TV sets, computers, video game devices etc.), reduced time when a child is outdoors or goes in for sport [13]. In our study the percentage of children with sedentary lifestyle was 81,3% (their physical activity was less than 0,5 hour a day). Practically every third child (29,7%) was sitting in front of the computer and/or TV set over 3 hours a day.

The introduced anamnestic questionnaire within the frame of the program of medical-social monitoring of obesity among schoolchildren on the level of our multidisciplinary health care institution in the future will allow longitudinal observation of the results of medical practice in this area. Detection of obesity predictors among children will promote timely organization of measures on prevention, formation of parents' approval of a well-balanced diet of their children, and changing family stereotype to a healthy lifestyle.

## CONCLUSIONS

The elaborated technique to predict development of an excessive body weight of children allows a general practitioner who gives primary medical aid by means of a simple method to find children with different degree of risk promoting development of obesity and/or an excessive body weight. Individual prognostication can be used as a screening test during annual preventive medical examinations of children receiving a substantiated prognosis of an excessive body weight development. By means of the individual prognostication individualized prophylaxis measures can be developed concerning prevention of obesity development and other diseases associated with an excessive body weight.

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ORIGINAL ARTICLE

# ROLE OF PRE-CESAREAN SECTION CEFOTAXIME IN AMELIORATED POST-CESAREAN COMPLICATION

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**ABSTRACT**
**The aim**: The purpose of this study was to examine the efficacy of cefotaxime before and after skin incision in avoiding post-operative infection complications in caesarean section women, also evaluation the efficacy of cefotaxime in reducing post-caesarean section complications.
**Materials and methods**: We conducted 150 women who undergoing caesarean section in the Obstetrics & Gynecological Department, Babylon government from January, 2021 to March, 2021. The caesarean operations were done by using standard protocols. Each patient was examined daily and post-operative infectious. Women were randomly divided into three groups; each group contains 50 women; Group 1: (control) given normal saline 12 hr. before and after skin incision. Group 2 (pre-operation antibiotic): given single dose of cefotaxime 1 g intravenously 12 hr. before skin incision, and Group 3 (post-operation antibiotic): given single dose of cefotaxime 1 g intravenously 12 hr after operation.
**Results**: The outcome measures were post-operative febrile morbidity, healing period and urinary tract infections, in addition to socioeconomic state of each woman.
**Conclusions**: cefotaxime pre-cesarean section could ameliorate post-operative problems such as infection of surgical wound, febrile, and urinary tract infections.

**KEY WORDS:** cefotaxime, cesarean section, wound infection, surgical site infection, and post-operative febrile

## INTRODUCTION
Cesarean section SCs (laparotomy) is surgery to deliver a baby. The baby is taken out through the mother's abdomen, the first cesarean documented occurred in 1020 AD [1]. SCs are planned and sometime occur when there are unexpected delivery problems [2]. SCs were done when there are some reasons include health mother problems, multiple fetuses, abnormal size or position of the fetus, fetus health problem, and labor is not moving alone [3]. One of major risk factor for postpartum infection is Cesarean section. About 22% of live births in Western countries were by cesarean section and as compare the women that undergo cesarean section were 5–20 folds greater than normal vaginal birth Women [4]. Complicationsoccur after cesarean such asInfectionwhich consider as important factorfor maternal damage and may cause elongation of hospitalization [5]. The procedure is relatively safe for both the mother and the infant. Nonetheless, it is a large operation with dangers. A C-section also takes longer to recover from than a vaginal birth. It can increase the chances of future pregnancies being challenging. Some women may have difficulty later attempting a vaginal birth. Despite this, many women are able to give birth vaginally following a cesarean section (VBAC) [6]. Every year, about 18.5 million cesarean sections are conducted around the world. Around 40% of countries have C-section rates of less than 10%, 10% have C-section rates of between 10% and 15%, and approximately 50% have C-section rates of

greater than 15% [7]. Since 1985, the international medical community has deemed a C-section rate of 10-15 percent to be desirable. C-sections have been more common in both developed and underdeveloped countries since then. Infection of the wound (common) – redness, swelling, growing discomfort, and drainage from the wound are all possible complications after caesarean section [8]. Fever, abdominal ache, irregular vaginal discharge, and excessive vaginal bleeding are frequent symptoms of infection of the uterine lining a lot of blood (uncommon). Deep vein thrombosis (DVT) is a clotting of the blood vessels in the legs (rare) a problem with your bladder or the tubes connecting your kidneys and bladder (rare) [9]. The main pregnancy hormones are estrogen and progesterone. Estrogen it is generated in the ovaries in most cases. The placenta also produces it throughout pregnancy to aid in the maintenance of a healthy pregnancy. During pregnancy, estrogen levels rise, allowing the uterus and placenta to function properly as transport nutrients, support the developing infant, and enhance vascularization (the creation of blood vessels) [10]. Estrogen is also thought to play a role in the development and maturation of the fetus. Estrogen levels rise gradually throughout pregnancy, peaking in the third trimester. Some of the nausea associated with pregnancy may be caused by the rapid increase in estrogen levels during the first trimester. It is important for the development of milk ducts, which enlarges the breasts, during the second trimester [11]. The state of fertilization

and development for one or more babies within a woman's uterus is known as pregnancy. Pregnancy is defined as the period of gestation that begins with the fertilization of an egg and ends with the birth of a child. As in the case of twins or triplets, a pregnancy might be many gestations. Childbirth normally happens at 38 weeks after conception, or around 40 weeks following the last normal menstrual cycle [12]. Pregnancy is split into three trimesters, each of which generally corresponds to a developmental stage. During the first trimester, the chance of spontaneous abortion (miscarriage) is higher, and it decreases in the second and third trimesters. During the second trimester, the fetus' growth and development may be more easily observed, but in the third trimester, a fetus is normally viable (though it may require medical intervention) [13]. Antimicrobials are frequently prescribed prophylactically in Obstetrics and Gynecology for pre and post-operative procedures caesarean section or to treat ongoing infection such as vaginitis, pelvic inflammatory, sexually transmitted diseases and urinary tract infections [14]. The appropriate use of antimicrobials in women of childbearing age is critical since it impacts both the women and their progeny. Antibiotics used randomly may result in the emergence of drug-resistant organisms [15]. The World Health Organization defines drug use as "the marketing, distribution, prescription, and use of pharmaceuticals in society, with a focus on the medical, social, and economic effects". A drug use study is one that aims to quantify and qualitatively describe the use of a certain drug, including the drug's class, indications, duration of treatment, dosage, preceding or concomitant therapies, and compliance [16]. Infections at the surgical site are a typical side effect of obstetric and gynecological treatments. Antimicrobial prophylaxis for caesarean section has been found to reduce postoperative morbidity, hospitalization costs, and length of stay. In the above scenario, antimicrobial use is unavoidable, but it should be limited. The proper administration of these treatments can help to avoid post-partum infection in both the mother and the baby, as well as lower the risk of unpleasant drug reactions. The goal of this study was to see how antimicrobials were used in 150 women who had a caesarean section [17]. When compared to women who give birth vaginally, women who have a caesarean section have a higher risk of infection. These infections can be transmitted by the urine, surgical incisions, or the womb lining (endometritis). Infections can be dangerous, resulting in a pelvic abscess or a blood infection, for example. They can, on rare occasions, result in the death of a mother, especially in low-resource circumstances. To prevent infection, good surgical methods, as well as the use of skin antiseptics and the administration of antibiotics prior to the caesarean section, are essential [18]. Cefotaxime (CLAFORAN) is a parenterally administered semisynthetic broad range cephalosporin antibiotic. It's a prescription medication that's used to treat symptoms of Gonococcal Urethritis or Cervicitis, Rectal Gonorrhea, bacterial infections, and as surgical infection prevention. Cefotaxime can be taken alone or in combination with other drugs [19]. Cefotaxime

belongs to the Cephalosporin, 3rd Generation medication class. Depending on the concentration and diluents used, cefotaxime solutions range from very pale yellow to light amber. Cefotaxime can cause serious side effects such as hives, difficulty breathing, and swelling of the face, lips, tongue, or throat, severe stomach discomfort, watery or bloody diarrhea, skin rash, bruises, extreme tingling, and numbness. The state of fertilization and development for one or more babies within a woman's uterus is known as pregnancy. Pregnancy is defined as the period of gestation that begins with the fertilization of an egg and ends with the birth of a child. As in the case of twins or triplets, a pregnancy might be many gestations. Child birth normally happens at 38 weeks after conception, or around 40 weeks following the last normal menstrual cycle.

## THE AIM

The purpose of this study was to examine the efficacy of cefotaxime before and after skin incision in avoiding post-operative infection complications in caesarean section women, also evaluation the efficacy of cefotaxime in reducing post-caesarean section complications.

## MATERIALS AND METHODS

### STUDY DESIGN

Our study was a conducted 150 women who undergoing elective caesarean section in the Obstetrics & Gynecological Department, Babylon government from 1/ 2021 to 3/ 2021. The caesarean operations were done by using standard protocols. Each patient was examined daily and post-operative infectious. Women were randomly divided into three groups with 50 women in each group. Group 1: (control) received normal saline 12 hr. before and after skin incision. Group 2 (pre-operation antibiotic): administered single dose of cefotaxime 1 g intravenously 12 hr. before skin incision, and Group 3 (post-operation antibiotic): administered single dose of cefotaxime 1 g intravenously 12 hr. after operation . The outcome measures were post-operative febrile morbidity, healing period and urinary tract infections, in addition to socioeconomic state of each woman.

### FOLLOW UP PHASE

The follow up was performed in the next day after operation, Fever (temperature & recurrent > 38 C°). After two days, begin the other investigations such as UTI and wound infection. Tenth day post-operative, the patients come back to clinic obstetrician to examine the wound healing process, infections and other markers of study.

### STATISTICAL ANALYSIS

We used mean ± SEM to express the data and One-way ANOVA $P < 0.05$ followed by Tukey's post hoc to find the difference in means of temperature, healing period, and UTI

between different groups. The descriptive statistics of categorical variables were presented by counts and percentage. The association between age groups, Resident, Occupation, and education was analyzed by application of chi square ($x^2$) test was used as appropriate at level of significance α = 0.05. All statistical analyses were applied using SPSS 26.0 for Windows and the graphs were draw by GraphPad Prism software v8.0.2.

**Table I.** Demographics properties of the three groups P-value < 0.05

| Age | | |
|---|---|---|
| | Frequency | Percent |
| 20-30 years old | 84 | 56.0 |
| 31-40 years old | 48 | 32.0 |
| 41-50 years old | 18 | 12.0 |
| Total | 150 | 100.0 |
| **Resident** | | |
| Urban | 24 | 16.0 |
| countryside | 126 | 84.0 |
| | 150 | 100 |
| **Occupation** | | |
| Housewife | 75 | 50.0 |
| Employed | 75 | 50.0 |
| Total | 150 | 100.0 |
| **Education** | | |
| Read and write | 36 | 24.0 |
| Basic & Secondary | 63 | 42.0 |
| University | 51 | 34.0 |
| Total | 150 | 100.0 |

# RESULTS

## DEMOGRAPHIC DATA
Table (I) was representing the demographic data of the three groups, we found that there is no significant difference (p>0.05) between three groups in age, occupation, education, and residence with

## TEMPERATURE
We noticed that the temperature was high in both control and post-operation antibiotic groups while the pre-operation antibiotic group showed low temperature. Also, we notice that there is insignificant difference (p > 0.05) between control group and post-operation group while there is significant difference (p<0.05) between post-operation antibiotic group and both control and post-operation antibiotic groups, see tables (II), (III), and figure (1).

From this figure, it was noticed that the highest temperature was notices in control and post-operation groups while the lowest one was seen in pre-operation group

## HEALING PERIOD
We noticed that the healing period was long in both control and post-operation antibiotic groups, while the pre-operation antibiotic group showed short. Also, we notice that there is insignificant difference (p > 0.05) between control group and post-operation group while there is significant difference (p<0.05) between post-operation antibiotic group and both control and post-operation antibiotic groups, see tables (IV), (V) and figure (2).

The longest period was notices in both control and post-operation groups while the shortest one was seen in pre-operation group.

**Table II.** the fever degree in C° among the three groups (P < 0.05)

| Groups | Mean ± Std. Error | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Control (No antibiotic) | 38.062 ± 0.137 | 0.971 | 37.785 | 38.338 |
| Pre-operation antibiotic | 36.960 ± 0.125 | 0.884 | 36.708 | 37.211 |
| Post-operation antibiotic | 38.090 ± 0.113 | 0.801 | 37.862 | 38.317 |

**Table III.** Multiple Tuky Comparisons analysis among months

| Group | No antibiotic | Pre-operation antibiotic | Post-operation antibiotic |
|---|---|---|---|
| Control (No antibiotic) | | * | |
| Pre-operation antibiotic | * | | * |
| Post-operation antibiotic | | * | |

Results represent difference between means of months *significant difference (P < 0.05)

**Table IV.** healing periods among the three groups (P < 0.05)

| Groups | Mean ± Std. Error | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Control (No antibiotic) | 2.3200 ± 0.1007 | 0.7125 | 2.1175 | 2.5225 |
| Pre-operation antibiotic | 1.7600 ± 0.110 | 0.884 | 1.5565 | 1.9635 |
| Post-operation antibiotic | 2.2800 ± 0.0634 | 0.776 | 2.0573 | 2.5027 |

**Fig. 1.** the different in temperature among the three groups.



**Fig. 2.** the different in healing period among the three groups.
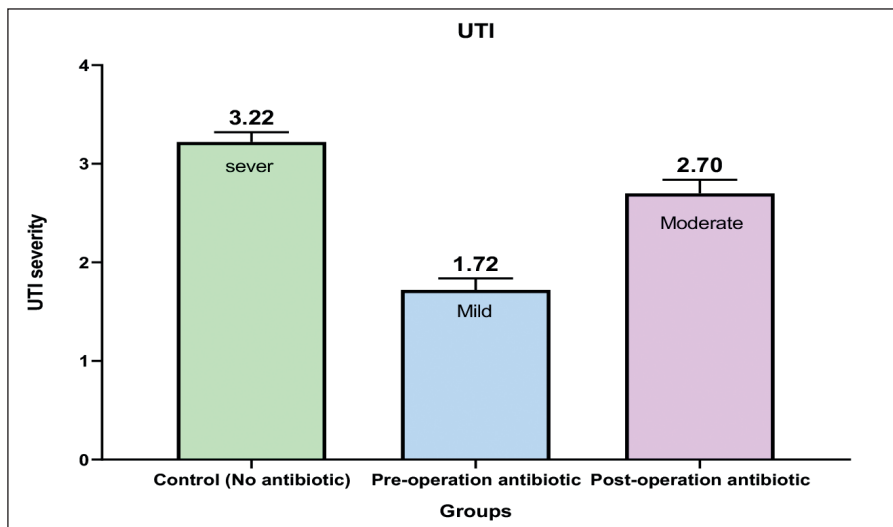


**Fig. 3.** the different in temperature among the three groups

## URINARY TRACT INFECTION

We noticed that the UTI was sever in control and moderate in post-operation antibiotic groups while the pre-operation antibiotic group showed mild UTI. Also, we notice that there is significant difference (p<0.05) between pre-opera-tion antibiotic group and both control and post-operation antibiotic groups, see tables (VI), (VII) and figure (3).

The severity UTI was notices in control and moderate one in post-operation groups while in pre-operation group was mild.

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**Table V.** Multiple Tuky Comparisons analysis among months

| Group | No antibiotic | Pre-operation antibiotic | Post-operation antibiotic |
|---|---|---|---|
| Control (No antibiotic) | | * | |
| Pre-operation antibiotic | * | | * |
| Post-operation antibiotic | | * | |

Results represents difference between means of months *significant difference (P < 0.05)

**Table VI.** the healing periods among the three groups (P < 0.05)

| Groups | Mean ± Std. Error | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Control (No antibiotic) | 3.220 ± 0.100 | 0.708 | 3.0187 | 3.4213 |
| Pre-operation antibiotic | 1.720 ± 0.117 | 0.884 | 1.4830 | 1.9570 |
| Post-operation antibiotic | 2.7000 ± 0.137 | 0.9741 | 2.4231 | 2.9769 |

**Table VII.** Multiple Tuky Comparisons analysis among months

| Group | No antibiotic | Pre-operation antibiotic | Post-operation antibiotic |
|---|---|---|---|
| Control (No antibiotic) | | * | * |
| Pre-operation antibiotic | * | | * |
| Post-operation antibiotic | * | * | |

Results represent difference between means of months *significant difference (P < 0.05)

## DISCUSSION

In the absence of post-operative morbidity, Caesarean deliveries spend an average of two days in the ward. Those who have difficulties may need to stay on the island for up to twice as long abdominal wounds, as has been discovered in prior investigations. Endometriosis, infection, fever, and urinary tract infection are all symptoms of endometriosis. The most serious post-surgery complication any type of intervention. As a result, reducing these difficulties will hasten recoveryas well as reducing the time of hospitalization. Maternal features in a statistically significant difference obstetrical history and contemporary obstetrical history are both important. According to the findings, the women 50% were housewives, and about 42% had basic skills and secondary education, also, we found the most of women were from country side regions. The majority of women, housewives who had cesarean sections, but she presented that countryside women made up the bulk of the population. In Sweden, [13] the authors found that the number of cesarean sections grew as women's lives progressed in countryside regions. Antimicrobial prophylaxis is also suggested. All cesarean deliveries are advised, and these lead tominimize the occurrence of fever and endometriosis. The administration of perioperative cefotaxime during cesarean birth lowered the incidence of fever, urinary tract infection, and wound infection, according to our findings. These outcomes agree with [19], who came at the same conclusionprophylaxis given 12 hours before to cesarean deliverylowers the incidence of postpartum fever and wound infection infected. According to Hofmeyr et al. (2010), [14] using perioperative antibiotics reduced the risk of postpartum fever by 75%, also cefotaxime was given before antibiotics. The urinary tract infection and

postpartum fever were reduced as a result of the procedure infected. Kawakita T, et al., 2017 [20] came to a conclusion delivery of an antibiotics treatment with a broad breadth appears to be linked to a decrease in post-cesarean births infection in the mother.

## CONCLUSIONS

Cefotaxime pre-cesarean section could ameliorate post-operative problems such as infection of surgical wound, febrile, and urinary tract infections.

## RECOMMENDATIONS

It was suggested based on the findings of this investigationthatintroducing wound care education to the public. The nurse believes that mothers have an important part in wound healing.As a result; there should be some encouragement from the top. This measure should be followed by nurses and doctors to help reduce the number of deaths found infection caused by C.S. Antimicrobial prophylaxis is advised for allcesarean deliveriesand that prophylaxis should be usedgiven at least 30 minutes before the start of the eventCesarean section is a surgical procedure that is used to deliver a baby, a single dosage for cesarean delivery prophylaxis. An antibiotic with a specific target, such as a first-generation cephalosporinis the first-line antibiotic of choice, unlesssignificant drugallergies are present.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

# PECULIARITIES OF THE SOURCES OF ORIGIN AND MORPHOGENESIS OF THE HUMAN MANDIBLE

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**ABSTRACT**
**The aim:** To determine the sources and terms of origin, developmental peculiarities and dynamics of ossification of the mandible during the prenatal period of human ontogenesis.
**Materials and methods:** The research was carried out on the specimens of 30 embryos, 30 pre-fetuses and 60 human fetuses at the period from the 9th to the 12th weeks of the intrauterine development, which were studied by microscopic examination. Three-dimensional computer reconstructions of the human pre-fetal head were made.
**Results:** During the 7th week of development the maxillary processes maximum approach the lateral and medial nasal ones; in pre-fetuses 20,0 mm of PCL they join the frontal spindle forming the facial structures (upper jaw and lip, vestibule of the oral cavity, rudiments of dental laminas, and rudiments of dental buds in its distal portions). Osteogenous islets, rudiments of the mimic and masticatory muscles, blood vessels are formed. During the 8th week of development the osseous tissue of the mandible is formed, the alveolar processes are formed. The oral and nasal cavities are isolated in 9-10-week pre-fetuses, the mass of the osseous tissue increases in both jaws, the enamel organs are detached, the angles and rami formed by the hyaline cartilaginous tissue of the mandible are determined, the rudiments of the temporomandibular joints are already seen. During the 11th week of development the osseous base of both jaws become formed. Till the end of the 12th week the osseous tissue begins to replace the hyaline cartilage of the mandibular rami, and the articular heads are formed in the portion of their proximal ends.
**Conclusions:** The mandible in its development is known to be characterized by intra-cartilaginous formation of the bone which starts from the ends of the cartilage gradually displaced by the osseous tissue. It is indicated that both jaws in pre-fetuses 37,0 and 42,0 mm of PCL are presented by the typical cartilaginous tissue, and in pre-fetuses 45,0-50,0 mm of PCL the osseous tissue is already available replacing the cartilaginous one.

**KEY WORDS:** mandible, morphogenesis, origin, ossification, ontogenesis

## INTRODUCTION

Learning the sources, terms, chronological sequence of morphological transformations, finding critical periods and developmental peculiarities of the anatomical structure of the stomatognathic system during the prenatal period of human ontogenesis are relevant areas of morphological studies promoting solution of an important medical-social issue – improvement of the methods of prevention, early diagnostics and effective surgical correction of congenital defects and treatment of the acquired diseases of the human mandible. Morphological description of the maxillofacial structures and peculiarities of development of the mandible in particular, does not keep pace with up-to-date requirements of practical medicine. Usually to investigate these issues experimental models are used (laboratory animals) [1]. The methods of development and analysis of two-dimensional reconstructions are applied at the late stages of the craniofacial human development [2]. The data concerning regulations and peculiarities of mandibular morphogenesis during the prenatal period of human ontogenesis are insufficient. Certain fragments of research are known – those concerning morphogenesis of the mandible in human pre-fetuses [3]. Clear understanding of etiopathogenesis of congenital pathology of the human body organs and systems is based on comprehensive mor-

phological examinations of peculiarities of the anatomical structures in the dynamics of the intrauterine development. Embryogenesis of the mandible differs by the formation of Meckel's cartilage followed by its involution and the processes of osteogenesis. Detection of chronological order of mandibular transformations at the early period of ontogenesis will promote development of morphological criteria of the norm, and improve diagnostic algorithms in interpretation of examination of human fetuses.

## THE AIM

The aim was to determine the sources and terms of origin, developmental peculiarities and dynamics of ossification of the mandible during the prenatal period of human ontogenesis.

## MATERIALS AND METHODS

The research was carried out on the specimens of 30 embryos, 30 pre-fetuses and 60 human fetuses at the Municipal Medical Institution «Chernivtsi Morbid Anatomy Bureau» according to the agreement on collaboration.

The investigations were performed keeping to the major regulations of the Resolution of the First National Congress
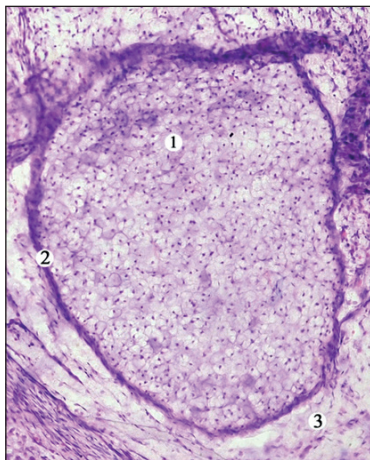
**Fig. 1.** Transverse section of Meckel's cartilage of the human embryo 13,5 mm of PCL. Staining with hematoxylin and eosin. Microphotograph. Magnification: 80x. Signs: 1 – Meckel's cartilage; 2 – perichondrium; 3 – mesenchyme.
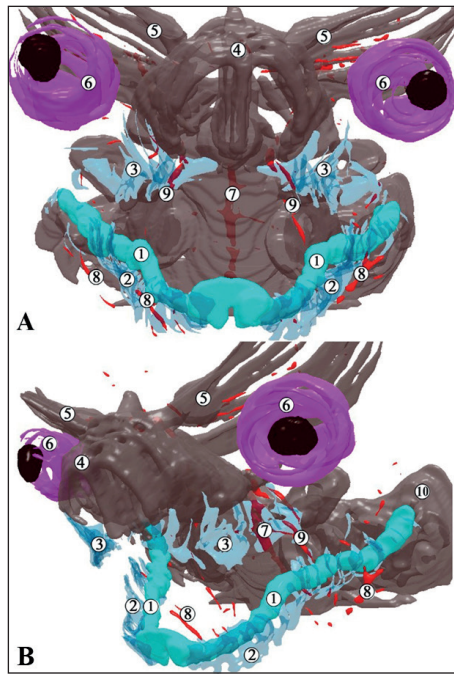


**Fig. 2.** Three-dimensional computer reconstruction of the human pre-fetal head 19,0 mm of PCL. A – anterior projection, Б – left anterior-lateral projection. Magnification: 15x. Signs:
1 – Meckel's cartilage; 2 – foci of mandibular osteogenesis; 3 – foci of the maxillary osteogenesis; 4 – nasal capsule; 5 – rudiment of the cranial bones; 6 – eyeballs; 7 – basilar artery; 8 – inferior alveolar artery; 9 – maxillary artery; 10 – auricular cartilage.
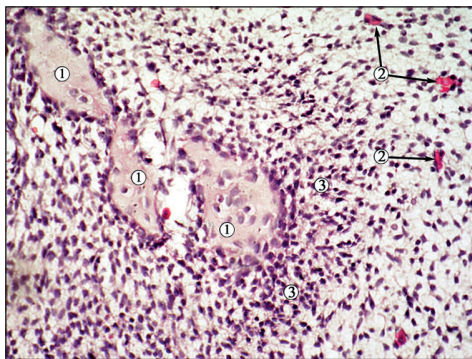


**Fig. 3.** Histological section of the pre-fetal mandible 18,0 mm of PCL. Staining with hematoxylin and eosin. Microphotograph. Magnification: 190x. Signs:
1 – osteogenous islets, 2 – condensed mesenchyme around the osteogenous islets, 3 – blood islets.



**Fig. 4.** Frontal section of the right mandible of the human pre-fetus 25,0 mm of PCL. Staining with hematoxylin and eosin. Microphotograph. Magnification: 50x. Signs:
1 – Meckel's cartilage; 2 – rudiment of the mandible; 3 – skin.
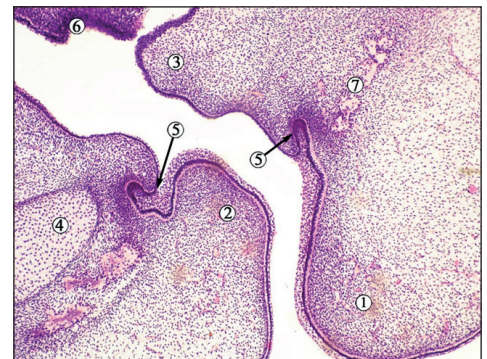


**Fig. 5.** Fragment of head section of the human pre-fetus 23,0 mm of PCL. The vestibule of the oral cavity lines the stratified epithelium. Staining with hematoxylin and eosin. Magnification: 30x. Signs:
1 – upper lip; 2 – lower lip; 3 – lateral palatine lamina; 4 – Meckel's cartilage; 5 – dental laminas; 6 – tongue; 7 – osteogenesis foci.

on Bioethics «General Ethic Principles of Experiments on Animals» (2001), ICH GCP (1996), the European Union Convention on Human Rights and Biomedicine (04.04.1997), and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (18.03.1986), the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (1964-2008), EU Directives №609 (24.11.1986), the Orders of the Ministry of Health of Ukraine № 690 dated 23.09.2009, №944 dated 14.12.2009, № 616 dated 03.08.2012.

## RESULTS

At the beginning of the 6th week of the intrauterine development (embryos 8,0-11,0 mm of the parieto-coccygeal length (PCL)) the anlage of Meckel's cartilage is clearly determined. Its central part is formed by oval cells densely packed. These cells are visually smaller than those surrounding cartilages (Fig. 1). They present a distinctive center for the beginning of the cartilage formation. The perichondrium begins to form along the periphery of Meckel's cartilage at the end of the 6th week of the intrauterine development.

Osteogenous islets are found in embryos 10,0-11,0 mm of PCL (the middle of the 6th week of the intrauterine development). These are the areas of mesenchyme hardening located on both sides of the cartilaginous mandibular anlages. The cellular elements in their content are characterized by other forms of cells and nuclear-cytoplasmic correlation in them. The degree of intensity of the osteogenous anlages

**Fig. 6.** Frontal section of the mandible of the human pre-fetus 35,0 mm of PCL. Staining with hematoxylin and eosin. Microphotograph. Magnification: 50x. Signs:
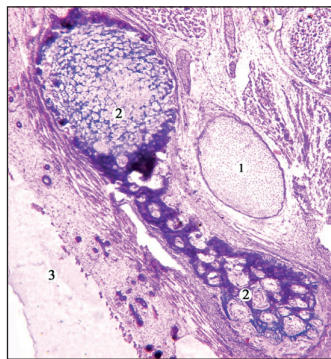1 – Meckel's cartilage; 2 – mandible rudiment; 3 – rudiments of teeth; 4 – tongue; 5 – mandibular-sublingual muscles; 6 – submental-sublingual muscles; 7 – submental-lingual muscles; 8 – anterior ventricles of the digastric muscles; 9 – sublingual glands; 10 – lingual arteries.
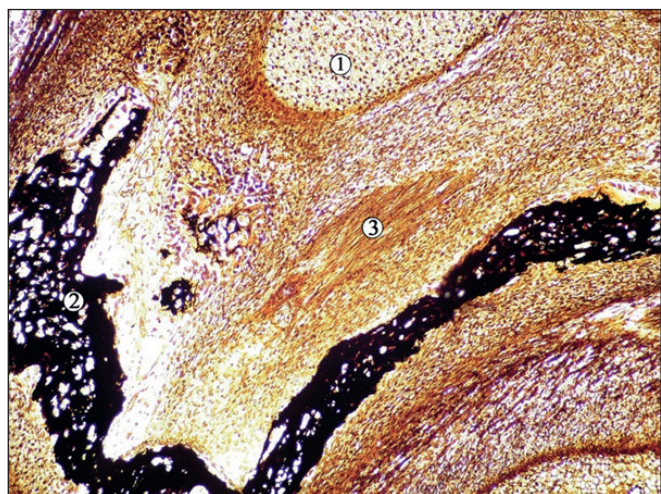


**Fig. 7.** Fragment of the mandible of the human fetus 55,0 mm of PCL. Silver impregnation. Microphotograph. Magnification.: 50x. Signs:
1 – Meckel's cartilage; 2 – osseous tissue trabecule; 3 – muscular elements.

decreases in the distal direction, and they are lacking in the areas of ventral extremities of Meckel's cartilage.

At the beginning of the pre-fetal period of the intrauterine development the submental nerve is detected close to the inferior border of Meckel's cartilage in the point of the primary ossification center of the mandible. Meckel's cartilages are delimited along the median line in the area of the chin by a thin mesenchyme layer.

At the end of the 7th week of intrauterine development (pre-fetuses 17,0-22,0 mm of PCL) the rudiment of the mandible is found externally from Meckel's cartilages occurring from the adjacent mesenchyme. A small concavity of the cartilage is seen into the center of the primary ossification of the mandible, followed by its ossification along the whole cartilage. At the end of the 7th week of intrauterine development mandible ossification occurs not only distally

from the primary center, but in the submental area as well. Due to this process Meckel's cartilage becomes surrounded by the bone along the anterior and posterior surfaces (Fig. 2). At the same time, the process of impression of the dental lamina in the space between the cartilage and anterior bony surface of the mandible is observed.

Special attention is drawn to the fact that the foci of osteogenesis localized externally from Meckel's cartilage in the shape of separate islets with clear signs of mineralization are found among the structures of the mandibular rudiments more clearly than in the objects of preliminary stages. This mineralization becomes especially visible at the end of the 7th week of the intrauterine development. Mineralized islets of the osseous tissue are surrounded by osteoblasts closely adjacent one to another. Their shape is various – from oval to trapezoid (Fig. 3).

Comparing the morphology of certain osteogenic islets our attention was drawn to the fact that they differed in the course of their development by their size, number of cells in the unit of volume of the intercellular matrix, degree of mineralization of osteogenesis centers, which is indicated by the difference of the tinctorial properties of their structures.

Mineralization of osteogenous islets was found to occur nonsynchronous repeating the dynamics of the processes of formation of the mandibular processes. We have found that those areas located closer to Meckel's cartilage are stained more intensely. We consider it is associated with reciprocal relations established between the structures of Meckel's cartilage participating in the formation of the mandible, and osteogenous components in the process of differentiation. Compact mesenchyme participating in osteogenesis is located around osteogenous islets.

The nuclei of osteoblasts are oval by shape. They are most often located eccentrically, dislocating to the apical pole, and the cytoplasm demonstrates pronounced basophilia in different degrees.

The cells located inside of the osteogenous islets become of an elongated stellate shape with various numbers of short processes. The cytoplasm of these cells is stained with the major dyes less intensively than in the area surrounding the islets of osteoblasts. They are located one by one in the so-called lacunas which outlines imitate the shape of cells. Due to the action of a fixing agent the sizes of cells become smaller than lacunar formations, therefore, light colorless cavities are found around them. Considering a specific shape of such cells isolated one from another by intercellular substance, they may be called osteocytes on their different stages of maturation.

Therefore, on the 7th week of the intrauterine development differentiation of structures participating in the formation of the maxillofacial apparatus occurs more rapidly in comparison with the previous stage of development. Osteogenesis is more active in the mandible. The vestibule of the oral cavity is formed, dental laminas are laid, and till the end of the 7th week dental germs are laid down. The mimic and masticatory muscles are differentiated.

During the 8th week of the intrauterine development (pre-fetuses 21,0-30,0 mm of PCL) further ossification of

the mandible occurs. It becomes visible in the area of its rami (Fig. 4).

Meckel's cartilage begins to lose its clear outlines and smooth surfaces, and the distance between the posterior extremities of cartilages enlarges. The cells of the cartilage become vacuolated and lose their nuclei. Ossification process spreads over all the areas of the mandible. Ossified rami of the mandible are the places for attachment of the masticatory muscles, they are located on both sides from the cartilage, and the base of the mandible surrounds it from below and from the side. The body of the mandible becomes U-shaped.

The islets of the osseous tissue are seen laterally from Meckel's cartilage. These islets in comparison with similar formations in the pre-fetuses of the 7th week of the intrauterine development become larger in size at the expense of their growth on the one hand, and on the other – at the expense of fusion between themselves. As a result, the space between osteogenous islets filled by mesenchyme becomes vividly smaller. The foci of ossification spread along Meckel's cartilages both in the proximal and distal directions making up the bony basis of the mandible, and the rudiments of alveolar processes in the shape of grooves open into the side of dental gums in particular.

Similar to the previous stage of development the mesenchyme around the cartilages is characterized by more compact location cell in it, and around the islets of the osseous tissue.

The foci of osteogenesis in the mandible are found in the form of separate osteogenous islets of various size (Fig. 5). They are oxyphil stained, and differentiated osteoblasts are localized along the periphery. Single osteocytes separated one from another by the intercellular matrix, are located in the middle of them. Some of the osteogenous islets are on the initial stages of osteogenesis in the form of osteoid masses surrounded by the osteogenous cells. Cells are still absent inside of such formations. Oxyphilia in the centers of osteogenesis of the maxillary rudiments is less prominent than in the osteogenous islets of the mandible. Thus, morphological peculiarities and tinctorial properties of the intercellular matrix of the osteogenous rudiments are indicative of the heterochronic bone formation in the mandible and maxilla.

During the 9th week of the intrauterine development osteogenesis is implemented actively, and due to this process their bony basis is formed. The osseous septa in the mandible which are located from both the ventral-lateral sides from Meckel's cartilage approach each other in the distal direction. Though in the area of the chin similar to the extremities of Meckel's cartilage they remain divided by the layer of the connective tissue presented by the cellular elements located compact. Due to appositional growth the amount of the osseous tissue in the maxillofacial apparatus increases considerably, and therefore it participates in the formation of the shape of the facial portion of the head.

Morphological structural changes of the osseous tissue are characterized by general biological regularities of osteogenesis in both jaws, but similar to the previous stage of development ossification in the mandible occurs quicker. Thus, if the maxilla does not have compact consolidation of the osseous islets, the latter practically form an integral osseous structure of a trabecular type in the mandible. At the same time, a common feature for both jaws is that the osseous tissue increases in them by means of an appositional way at the expense of active proliferation of osteoblasts located on the surface of the osseous tissue, and their secretion of the intercellular substance components in which they gradually are embedded.

Morphology of the osteoblasts located in the periphery of the osseous basis of the jaws is rather variable. First of all, they are different by their shape. They may be oval and amorphous. Basophilia of their cytoplasm is diverse, which is associated with a different degree of their differentiation. In the course of embedding the cells into the intercellular substance they become of an elongate shape.

At this stage of development the rates of histogenetic formation take place in the mandible. One of the signs is more accelerated osteogenesis in it. Stromal elements of the red bone marrow are formed in the centers of its osseous basis, while it is not observed in the upper jaw.

The alveolar processes in the form of osseous laminas connected close to the base are formed in both jaws.

The distal extremities of the osseous laminas form alveolar grooves (Fig. 6). And the thickness and degree of development of the external laminas are more expressed in comparison with the internal ones. The grooves are filled with mesenchyme inside. Bot typical mesenchyme cells and those on the stage of their differentiation are found in its loosely located cells. The structures of the alveolar nerves with basophil staining are clearly seen by their morphological peculiarities between the mesenchyme cells located in the alveolar grooves.

During the 10th week of the intrauterine development the rates of differentiation of the hard and soft tissues of the human maxillofacial apparatus continue to increase compared to the previous stage of development. The upper jaw is modeled by the osseous tissue islets fused between themselves, and the hard basis of the mandible together with the osseous tissue are still formed by Meckel's cartilage.

Osseous rudiments of both jaws are of a typical structure peculiar for the rough fibrous osseous tissue. The signs of periosteum formation are found in some places of their periphery, the external and internal layers can be seen in it. Fuchsine stained collagen fibers are found in the external layer, osteoblasts oriented by their long diameters parallel to the surface of the osseous base are located in the internal layer.

The osseous tissue matrix is unevenly contrasted. Its peripheral area manifests oxyphilic properties, and the central one is stained by the common dyes. Lacunas of various shapes are seen in it. Osteocytes with cytoplasmic processes emerging from them are located in the lacunas. As a result of fixing agent action their bodies become considerably smaller, therefore unstained cavities are visualized around cells. They give the osseous tissue a porous view.

Fusion of the distal extremities of Meckel's cartilage in the area of the chin at the end of the 10th week of the intrauterine

development is a specific feature of the mandible development. The osseous formations located in the ventral-lateral position are directed forward coming closer together and are connected along the midline by means of the retention connective tissue ligament, which morphologic features are similar to the 9-week pre-fetuses.

Formation of the alveolar process continues along the whole osseous basis. The borders of its walls are directed to the side of dental rudiments and envelope them Y-shaped. The alveolar groove is filled with mesenchyme containing blood vessels and big trunks of the alveolar nerves.

The rami of the mandible are more visualized at this stage of development. They emerge from its proximal portions at an obtuse angle and decline from Meckel's cartilage cranially into the direction of the temporal bones where the areas of mesenchyme cells condensation are determined. They are rudiments of the heads of the temporomandibular joints.

Contrary to the osseous basis of the mandibular body which is directly formed from the mesenchyme, its rami are formed by the hyaline cartilage which is displaced by the osseous tissue later. Its intercellular matrix manifests pronounced basophilia.

During the 11-12th weeks of the intrauterine development the rami of the mandible continue to form. First they are formed by the hyaline cartilage, but to the end of the 12th week of the intrauterine development narrow deposits of the osseous tissue appear around the cartilage which differ from it by tinctorial features.

Meckel's cartilage is located to the middle from the osseous basis of the mandibular body. Space between it and the osseous tissue is filled with mesenchyme. This space is dilated in the proximal portion and narrowed to minimum in the distal direction.

Argyrophilic fibers are found in the connective tissue structures of the maxillofacial apparatus with silver impregnation. The centers of ossification are impregnated more intensively in comparison with other structures. The intercellular matrix of Meckel's cartilage is non-reactive, but the structural elements of the muscular tissue are found clearly (Fig.7).

Formation of the mandibular rami continues in the 12-week pre-fetuses. These rami emerge from the angles of the mandible in the dorsal-cranial direction. The hard base of the rami consists of the hyaline cartilage in the form of continuous bundles with relatively smooth borders. An amorphous component of the hyaline cartilage forming the mandibular rami and the heads of the temporomandibular joints demonstrate sharply basophilic properties. As far as the hyaline cartilage is replaced by the osseous tissue, tinctorial properties change into oxyphilic ones, and as a result the border between them is clearly visualized in the form of a broken line. Formation of the heads of the temporomandibular joints continues at this stage of development.

## DISCUSSION

We have found that at the end of the 5th week of embryogenesis isolation of Meckel's cartilage is observed in the mandibular rudiments. The cartilage makes up their hard base. Parallel to this process the foci of mesenchyme condensation located in the lateral direction from the cartilage anlages are determined. From the topographic point of view these foci correspond to osteogenous islets which become more apparent during further stages of embryogenesis, that is, during the 6-7 weeks of development. In this respect the data of our study correspond to the data of other researchers [4].

The foci of direct osteogenesis in the upper jaw appear a week later after the fusion of the maxillary processes with the nasal and medial frontal processes. The researchers of this issue state [5] that the foci of the membranous ossification in both jaws are clearly determined in the embryos of the 8th week of development stained with alizarin and cleared in xylene, the parietal-coccygeal length of these embryos is 23,5 mm. At the same time, there are several ossification centers in the upper jaw which develop from the heterogeneous anlages. Thus, its cutting part is formed of the material of the nasal passages, and the rami originate from the maxillary spindles of the mandibular arch. The osseous tissue of these maxillary rami undergoes ossification first, while in the cutting portion this process occurs later.

According to the literary data [6], the human embryos 12,5-13,0 mm of PCL already have the primary palate. It is known to be resulted from the fusion of the distal extremities of the palatine processes [7]. Other sources state that this process occurs during the 8th week of embryogenesis. During the fetal period starting from the 9th week of development [8], the proximal portions of the palatine processes continue to approach each other. Their fusion is over at the end of the 9th week of the intrauterine development (33,0 mm of PCL) resulting in the formation of the secondary palate. These data are confirmed by our investigations as well. Still other sources admit that this process only starts at the 7-8 week and is over during the 10th week [9].

According to the data of the scientific sources [10], the rudiments of the maxillary sinuses in the form of small hollow formations begin to isolate during the 9th week of the intrauterine development. Our studies found that during this period of development the alveolar groove is formed in the mandible. The wall of the groove is formed by the two osseous laminas: internal and external. And the internal osseous lamina first is thinner in comparison with the external one. Free borders of the groove open into the side of enamel rudiments and involve them as Y-shaped. The alveolar groove is filled with poorly differentiated mesenchyme in which alveolar nerves pass and blood vessels are formed that gradually join together, and finally contact the rami of the major alveolar vessels. The process of the alveolar groove formation in the upper jaw is lagging behind in comparison with the mandible.

The osseous formations are the most extensional near the base of the alveolar groves of both jaws. Their amorphous part along the periphery is poorly oxyphile stained, while the central part of the alveolar crests demonstrates besophilia, and morphology of the cellular elements is similar to that of the chondrocytes.

Chondroid is supposed to be the periosteal osseous tissue in the process of formation, and the cells contained in it are similar to those of cartilage by their morphological characteristics. They are modified osteocytes possessing a convergent likelihood with chondrocytes. In the process of differentiation that makes up the basis of embryonic histogenesis the cellular elements of one and the same type are considered to be able to undergo a number of specific qualitative changes. As a result of these changes the cellular elements are specialized to perform certain functions [11].

Our studies demonstrated that in pre-fetuses 40,0 mm of PCL (the end of the 10th – beginning of the 11th weeks of development) the longitudinal fusion of Meckel's cartilage in the mandible results in maximum approach of its distal extremities and their fusion in the area of the chin.

During 11-12th weeks of the intrauterine development paired processes are formed in the area of the proximal ends of the mandibular rami directed upwards: ventral coronary and dorsal condylar ones.

At this period the structures forming articular heads appear on the ends of the condylar processes. Certain researchers state that isolation of these structures in the form of mesenchyme condensation occurs much earlier – during the 8th week of the intrauterine development. Their further formation continues during subsequent 10-12 weeks in the form of rudiments of the hyaline cartilage which later will be replaced by the osseous tissue. Simultaneously with isolation of the articular heads the sockets of the joints begin to form as well. Their endesmal ossification continues as far as the articular heads are isolated. Meanwhile, even in 4-month human fetuses the temporomandibular joints are characterized by their incomplete structure. We did not find the formation of the temporomandibular joints during the 11-12th weeks of the intrauterine development either.

According to our data at this period the structure of the mandibular rami is mostly represented by the hyaline cartilage covered with a thin layer of the osseous tissue. The formation of the tissue occurs by means of appositional overlapping on the cartilaginous anlages modeling the rami. Therefore, the cartilage undergoes degenerative changes and is replaced by the osseous tissue.

Thus, contrary to the formation of the mandibular body, the formation of the osseous base of the mandibular rami results from indirect osteogenesis. And the data found in the course of our investigations correspond with the data of other researchers [12].

The rudiments of the mandibular rami in the distal direction join the osseous formations of its body. These formations from both sides envelope Meckel's cartilage in the form of an arch externally and approach one another in the area of the chin. Though, contrary to Meckel's cartilage they do not join together. Temporary compact connective tissue is formed between them at this period of time performing the role of a connective element at this stage of development. Further it will be replaced by the osseous tissue.

According to our findings, in the process of formation of the mandibular rami as far as their cartilaginous rudiments are replaced by the osseous tissue, the cartilaginous cells swell and enlarge in their sizes, their cytoplasm changes tinctorial properties becoming light and vacuolated. Glycogen is accumulated in it, and the nuclei undergo pyknotic changes and shrink. The major substance of the cartilage becomes harder and undergoes destruction. The mesenchyme grows into these places. A part of its cells are transformed into chondroclasts breaking down the cartilaginous tissue, and on its place mesenchyme cells are differentiated into the osteoblasts and osteocytes. Advance of this process is best observed in the areas of future articular heads.

The mandible in its development is known to be characterized by intra-cartilaginous formation of the bone which starts from the ends of the cartilage gradually displaced by the osseous tissue. It is indicated that [13] both jaws in pre-fetuses 37,0 and 42,0 mm of PCL are presented by the typical cartilaginous tissue, and in pre-fetuses 45,0-50,0 mm of PCL the osseous tissue is already available replacing the cartilaginous one. Although, we did not find enchondral osteogenesis of Meckel's cartilage in the objects examined.

## CONCLUSIONS

1. During the 7th week of development (pre-fetuses 14,0-20,0 mm of PCL) the maxillary processes maximum approach the lateral and medial nasal ones; in pre-fetuses 20,0 mm of PCL they join the frontal spindle forming the facial structures (upper jaw and lip, vestibule of the oral cavity, rudiments of dental laminas, and rudiments of dental buds in its distal portions). Osteogenous islets, rudiments of the mimic and masticatory muscles, blood vessels are formed.

2. During the 8th week of development the osseous tissue of the mandible is formed, the alveolar processes are formed.

3. The oral and nasal cavities are isolated in 9-10-week pre-fetuses (33,0-40,0 mm of PCL), the mass of the osseous tissue increases in both jaws, the enamel organs are detached, the angles and rami formed by the hyaline cartilaginous tissue of the mandible are determined, the rudiments of the temporomandibular joints are already seen.

4. During the 11th week of development the osseous base of both jaws become formed. Till the end of the 12th week the osseous tissue begins to replace the hyaline cartilage of the mandibular rami, and the articular heads are formed in the portion of their proximal ends.

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ORIGINAL ARTICLE

# PROTEOLYTIC ACTIVITY IN THE HEART OF RATS WITH HYPERHOMOCYSTEINEMIA

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**ABSTRACT**
**The aim:** To investigate the distribution of proteolytic activity and cytokine profile in the heart of rats with hyperhomocysteinemia.
**Materials and methods:** A total of 60 albino non-linear male rats was used in the study. Hyperhomocysteinemia was induced by intragastric administration of DL-homocysteine thiolactone. Total proteolytic activity was measured using casein as a substrate. To determine the activity of metal-dependent and serine proteases, ethylenediaminetetraacetic acid, and phenylmethylsulfonyl fluoride were used. The level of matrix metalloproteinases, tissue inhibitor of metalloproteinases-1, and cytokines was studied by enzyme-linked immunosorbent assay.
**Results:** It was found an increase in the total proteolytic activity in the heart of young, adult, and old animals. In addition, the redistribution of proteolytic activity was revealed – the portion of metal-dependent enzymes increased in all groups while the percentage of serine proteases decreased in the old animals with hyperhomocysteinemia. The state of mild inflammation, evidenced by the increased level of some pro-inflammatory cytokines, was found in the heart of young and old animals with hyperhomocysteinemia.
**Conclusions:** The pathogenesis of hyperhomocysteinemia is accompanied by a change in the proteolytic activity in the heart as well as a change in the cytokine profile.

**KEY WORDS:** hyperhomocysteinemia, proteolytic activity, cytokines

## INTRODUCTION

A number of studies have demonstrated that the elevated level of the amino acid homocysteine is an independent risk factor for cardiovascular diseases [1,2]. Moreover, a high serum homocysteine level is strongly associated with myocardial infarction, atherosclerosis, and thrombosis. Despite numerous scientific efforts, the exact mechanisms of pathological effects of increased homocysteine levels are not yet fully established. The fact that excess homocysteine is harmful to most tissues suggests that the harmful effects of homocysteine are mediated through common fundamental mechanisms. Homocysteine has been shown to induce both oxidative stress and inflammation [3,4]. Among the direct consequences of the accumulation of reactive oxygen species, as well as changes in the cytokine profile, are increased proteolysis and redistribution of proteolytic enzymes. Proteolytic homeostasis undoubtedly plays an important role in maintaining overall cell health. Therefore, the rate of proteolysis must be tightly controlled in order to prevent pathological consequences and the development of the disease. The activation of proteolytic enzymes in the heart is considered an important factor in the progression of heart disease. Given the crucial function of the extracellular matrix (ECM) in maintaining the appropriate structural integrity of the heart, abnormalities

in EMC metabolism can trigger changes in myocardial structure that ultimately affect function. Among the enzymes, that involve in the tissue remodeling processes are matrix metalloproteinases (MMPs) – a family of enzymes responsible for the breakdown of extracellular matrix, as well as basal membrane of the vessels [5]. Uncontrolled MMP activation can manifest as a variety of pathological conditions, including rheumatoid arthritis, tumor cell metastasis, periodontal disease, atherosclerosis, and heart disease.

## THE AIM

The current study was conducted to assess the distribution of proteolytic activity and cytokine profile in the heart of rats with hyperhomocysteinemia (HM).

## MATERIALS AND METHODS

### REAGENTS

Thiolactone D,L-homocysteine, tris(hydroxymethyl) aminomethane, o-phenylenediamine, hydrogen peroxide, ethylenediaminetetraacetic acid, phenylmethylsulfonyl fluoride, trichloroacetic acid, and casein were purchased from

Sigma-Aldrich (St. Louis, MO, USA). Anti-rat monoclonal antibodies to TNFα, INFγ, IL-1b, IL-4, IL-6, IL-8, IL-10, MMP-1, -2, -3, -8, -3/10, and TIMP-1 were purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade quality and available commercially.

## ANIMALS AND EXPERIMENTAL DESIGN

A total of 60 albino non-linear male rats was used in the study. All experiments on animals were performed in the compliance with international principles of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986). The study was approved by the Ethical Committee of Taras Shevchenko National University of Kyiv. The experiments were started after 7 days of animal acclimation in the animal facility of Taras Shevchenko National University of Kyiv, maintained under constant conditions of temperature ($22 \pm 3°C$), humidity ($60 \pm 5 \%$), and light (12 h light/12 h dark cycle). Standard rodent food and water were provided *ad libitum*. The animals of different ages were used in the current study – one-month-old rats that are corresponded to young animals; six-month-old rats that are corresponded to adult animals, and twenty-month-old rats that are corresponded to old animals. Hyperhomocysteinemia was induced by intragastric administration of DL-homocysteine thiolactone diluted in 1 % starch solution (100 mg·kg$^{-1}$ of body weight), ones per day for 28 days [6]. The control rats were received an equal volume of 1 % starch. HM development was confirmed by the high blood level of homocysteine (more than 15 µmol·L$^{-1}$). The level of homocysteine in the blood plasma was determined by enzyme-linked immunosorbent assay using the kit «Homocysteine EIA» (Axis-Shield, UK). On the 29th day since the start of the experiment, animals were killed. Thus, there were 3 experimental groups each of them consists of control rats (ten animals) and rats with HM (ten animals): 1) Group #1 (young animals); 2) Group #2 (adult animals); and 3) Group #3 (old animals).

## HEART SAMPLE PREPARATION

The heart was immediately collected after the animals have been killed. The tissue (1 g) was homogenized in 9 mL ice-cold 50 mM Tris-HCl buffer (pH 7.4) and further centrifuged at 12 000 g for 30 min at 4°C. The supernatant was collected and stored at 80°C for further biochemical analysis. The protein concentration was determined by the Bradford method [7].

## DETERMINATION OF TOTAL PROTEOLYTIC ACTIVITY, ACTIVITY OF METAL-DEPENDENT AND SERINE PROTEASES

Total proteolytic activity was measured using casein as

a substrate according to the method [8]. The activity of metal-dependent and serine proteases was assessed using corresponding inhibitors – ethylenediaminetetraacetic acid for the estimation of metal-dependent enzymes and phenylmethylsulfonyl fluoride for the estimation of serine proteases. Both inhibitors were used at the final concentration of 5 mM. The tested samples were preincubated with inhibitor for 30 min at 4°C and then the remaining enzyme activity was estimated using casein as a substrate.

## MMPS, TIMP-1, AND CYTOKINES IMMUNOASSAY

MMPs, tissue inhibitor of metalloproteinases (TIMP-1), and cytokines measurements were done by enzyme-linked immunosorbent assay (ELISA) according to the standard instructions [9]. ELISA plates were coated overnight at 4°C with samples of thyroid gland previously diluted with Tris–HCl buffer (pH 7.4) to obtained concentration of proteins 10 µg·mL$^{-1}$. After being washed, plates were blocked with 5 % nonfat dry milk for 1 h at 37°C and washed again. Next plates were incubated for 1 h at 37°C with specific primary antibodies against the cytokines (IFN-g, IL-1b, IL-12, IL-4, IL-10), MMPs (-1, -2, -3, -8, 3/10), and TIMP-1. Plates were washed and incubated for 1 h at 37°C with corresponding secondary antibodies conjugated to horseradish peroxidase. After washing, substrates (o-phenylenediamine and hydrogen peroxide) were added. The reaction was stopped by addition of 2.5 N H$_2$SO$_4$. Plates were read at 492 nm by a microplate reader (mQuant™, BioTek Instruments, Inc).

## STATISTICAL ANALYSIS

The data of biochemical estimations were reported as mean±SEM for each group (n = 10). Statistical analyses were performed using one-way analysis of variance (ANOVA). Differences were considered to be statistically significant when $p < 0.05$.

## RESULT

First, the total proteolytic activity in the heart of HM rats was assessed. The obtained results showed an increase in this parameter in the rats of all studied groups (Table I). The total proteolytic activity increased by 1.88-fold, 2-fold, and 2.23-fold in the heart of HM rats of Group #1, Group #2, and Group #3, respectively. The distribution of proteolytic activity in the heart of rats with HM was also assessed. It was found that HM is accompanied by an increase in the fraction of metal-dependent enzymes in the heart; the change in the level of metal-dependent enzymes was more pronounced in the group of old animals (22 % in control vs 58 % in HM rats) (Fig. 1).

The present findings showed an increase in the level of MMPs in the heart of HM rats of Group #1 – the level of MMP-1, -2, -3, and -3/10 was higher than the corresponding control values by 25 % (p<0.05) while the level of MMP-8 was increased by 15 % (p<0.05) (Table II). Analysis of MMP levels in the HM rats of Group #2 has revealed a less pronounced change in this parameter.
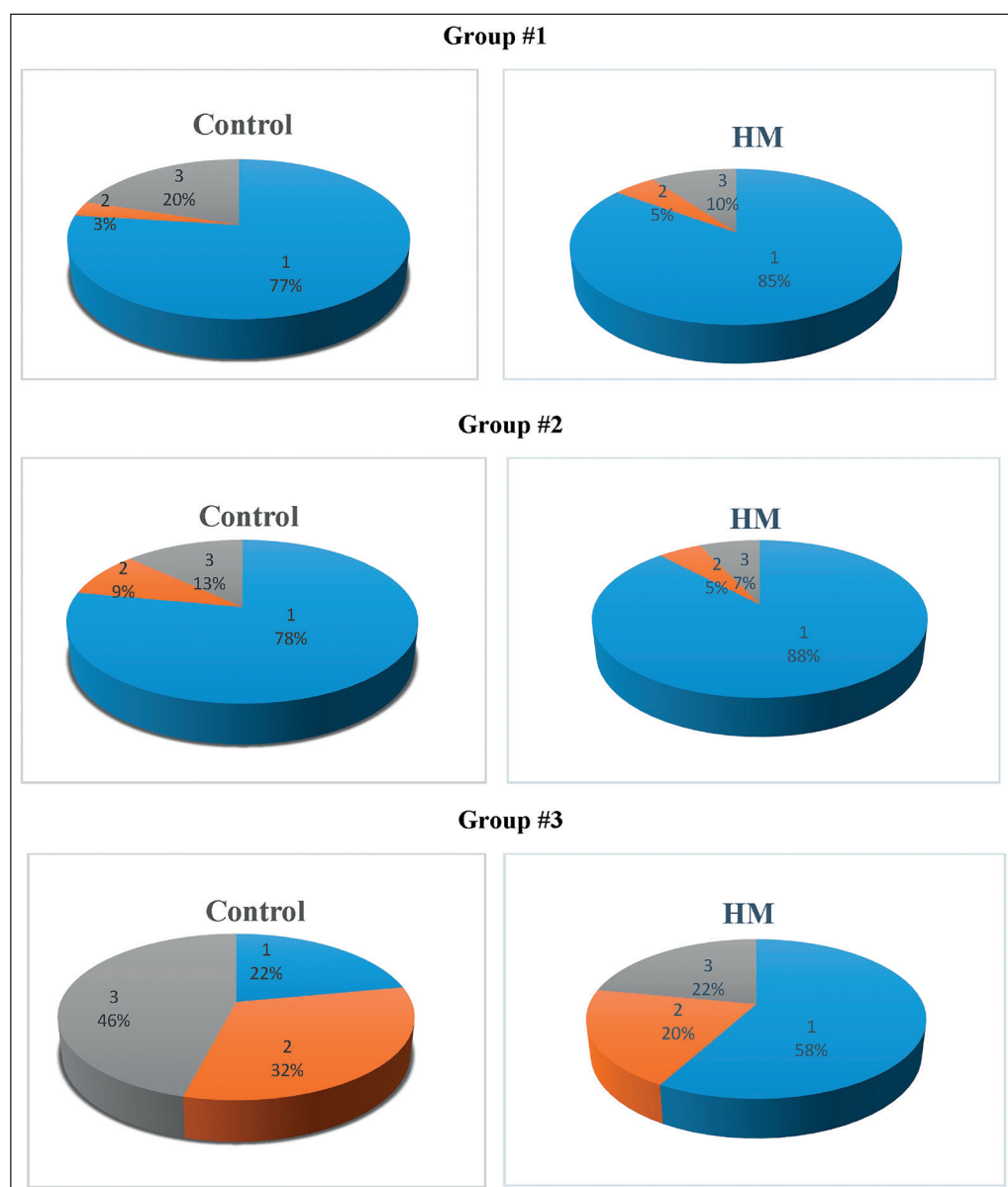
**Fig. 1.** The distribution of proteolytic activity in the heart of rats with hyperhomocysteinemia: 1 – metal-dependent proteases; 2 – serine proteases; 3 – other proteases

**Table I.** Total proteolytic activity in the heart of rats with hyperhomocysteinemia

| | Group #1 | | Group #2 | | Group #3 | |
|---|---|---|---|---|---|---|
| | **Control** | **HM** | **Control** | **HM** | **Control** | **HM** |
| rel.units·g-1 of tissue | 3.17±0.21 | 5.99±0.03* | 4.52±0.04 | 9.43±0.02** | 2.13±0.01 | 4.77±0.03*** |

Values are expressed as mean ± SEM (n = 10); *p<0.05 significantly different from the control of Group #1; **p<0.05 significantly different from the control of Group #2; ***p<0.05 significantly different from the control of Group #3

Thus, the level of MMP-1, -2, and -3/10 increased by 21 % (p<0.05), 17 % (p<0.05), and 15 % (p<0.05), respectively. In contrast, the levels of MMP-3 and MMP-8 were within control values. The most significant changes in the levels of MMPs in Group #3 were detected for MMP-8 – the level of this enzyme was 27 % (p<0.05) higher than in the corresponding control group. According to the obtained data, the level of TIMP-1 in the rats of Group #1 increased by 29 %. The opposite situation was observed in the rats of Group #2 and #3 – the level of inhibitor decreased by 35 % (p<0.05) and 7 % (p<0.05), respectively.

At the same time, the level of anti-inflammatory cytokines in the heart of HM rats was also increased. Thus, the levels of IL-4 and IL-10 increased by 42 % (p<0.05) and 14 % (p<0.05), respectively. Analysis of the cytokine profile in the rats of Group #2 has revealed an increase in the concentration of TNFα by 12 % (p<0.05); other cytokine levels were at the control values. The levels of TNFα and IL-6 in the heart of rats of Group #3 increased by 20 % (p<0.05); the levels of IL-1b and IL-8 were also higher than those in the control group. No significant changes in the level of anti-inflammatory cytokines IL-4 and IL-10 were detected in the rats of Group #3.

**Table II.** Level of MMPs and TIMP-1 in the heart of rats with hyperhomocysteinemia

| | | MMP-1 | MMP-8 | MMP-2 | MMP-3 | MMP-3/10 | TIMP-1 |
|---|---|---|---|---|---|---|---|
| | | rel. units·mg-1 of proteins | | | | | |
| Group #1 | Control | 80.0±4.0 | 95.0±4.7 | 180.0±9.0 | 140.0±7.0 | 180.0±8.5 | 170±8.0 |
| | HM | 100.0±5.0* | 110.0±5.5* | 225.0±9.5* | 175.0±6.5* | 230.0±9.5 | 220.0±8.0* |
| Group #2 | Control | 140.0±5.5 | 140.0±5.0 | 285.0±10.5 | 250.0±10.5 | 315.0±10.0 | 340.0±11.0 |
| | HM | 170.0±8.5** | 135.0±5.5 | 335.0±15.0** | 235.0±8.5 | 365.0±16.5** | 305.0±15.0 |
| Group #3 | Control | 160.0±6.0 | 165.0±6.0 | 315.0±10.5 | 265.0±10.0 | 350.0±15.5 | 360.0±16.0 |
| | HM | 190.0±8.5*** | 210.0±9.5*** | 345.0±12.5*** | 280.0±10.5 | 370.0±16.5 | 315.0±12.0*** |

Values are expressed as mean ± SEM (n = 10); *p<0.05 significantly different from the control of Group #1; **p<0.05 significantly different from the control of Group #2; ***p<0.05 significantly different from the control of Group #3

**Table III.** Level of cytokines in the heart of rats with hyperhomocysteinemia

| | | Pro-inflammatory cytokines | | | | | Anti-inflammatory cytokines | |
|---|---|---|---|---|---|---|---|---|
| | | TNFα | INFγ | IL-1b | IL-6 | IL-8 | IL-4 | IL-10 |
| | | rel.units·mg -1 of proteins | | | | | | |
| Group #1 | Control | 100.0±5.0 | 100.0±5.0 | 85.0±3.5 | 105.0±5.0 | 150.0±7.5 | 130.0±6.5 | 175.0±8.7 |
| | HM | 135.0±6.7* | 105.0±4.5 | 100.0±4.5* | 115.0±4.5* | 195.0±8.7* | 185.0±6.5* | 200.0±9.5* |
| Group #2 | Control | 165.0±5.5 | 140.0±6.5 | 140.0±6.5 | 155.0±6.5 | 300.0±10.0 | 265.0±8.5 | 290.0±14.5 |
| | HM | 185.0±8.0** | 135.0±6.5 | 130.0±6.0 | 150.0±7.5 | 295.0±10.0 | 250.0±9.5 | 300.0±13.0 |
| Group #3 | Control | 175.0±7.5 | 145.0±6.0 | 160.0±7.0 | 175.0±7.5 | 305.0±13.5 | 270.0±9.5 | 325.0±13.5 |
| | HM | 210.0±9.5*** | 155.0±6.0 | 185.0±8.5 | 210.0±7.5*** | 375.0±16.5*** | 265.0±8.0 | 345.0±15.0 |

Values are expressed as mean ± SEM (n = 10); *p<0.05 significantly different from the control of Group #1; **p<0.05 significantly different from the control of Group #2; ***p<0.05 significantly different from the control of Group #3

## DISCUSSION

Uncontrolled and excessive proteolysis may cause cardiac dysfunction and even cell death due to necrosis and/or apoptosis. In the current study, we have demonstrated that hyperhomocysteinemia affects proteolytic balance in the heart. This is manifested, first of all, in a significant increase in the total proteolytic activity and the activity of metal-dependent proteases. Taking into account the results on the distribution of proteolytic activity in the heart of rats with HM, particularly, a significant part of metal-dependent enzymes, it seems reasonable to analyze the level of MMP as the dominant part of metal-dependent enzymes present in the heart. The family of MMPs includes a number of zinc-containing endoproteases with different substrate specificity. In order to obtain a comprehensive assessment of the involvement of MMPs in the pathogenesis of HM, the level of MMPs belonging to different groups of enzymes was investigated. Our attention was focused on the collagenases (MMP-1, -8) that degrade fibrillar collagen types I, II, and III; the gelatinases (MMP-2) due to their ability to degrade type IV collagen in basement membranes, and the stromelysins (MMP-3, -3/10) which are active against a broad spectrum of ECM components, including proteoglycans, laminins, fibronectin, vitronectin [5,10]. The synthesis and degradation of collagen is a strictly balanced process, therefore, an increase in the level or/and activity of MMPs in the rats with HM can adversely affect the metabolism of collagen. Given that most of the myocardial collagen fibers consist of collagen types I and III, an increase in the level of MMP-1 and MMP-8 may be one of the reasons for structural and functional changes in the heart under HM. The situation is exacerbated in the case of older animals, for which a decrease in the production of collagen is a physiological process. Taking into account the role of MMPs in the development of cardiovascular diseases, our results can be considered as a poor prognostic criterion for those with HM.

Due to the strong proteolytic potential of MMPs, their concentration is usually maintained at a low level in physiological conditions. MMP activity may be regulated by different mechanisms. The binding of MMPs by endogenous inhibitors is one of the most effective ways to prevent the over-activation of MMPs [11]. Considering TIMP-1 suppresses the activity of most MSMs, the level of this inhibitor in the heart of HM rats was assessed. According to obtained data, an increased level of TIMP-1 in the heart of young rats with HM (Group #1) may indicate the activation of compensatory mechanisms aimed at reducing the level of active enzymes and maintaining homeostasis. In contrast, a decreased level of TIMP-1 and increased levels

of MMPs in older rats (Group #2 and Group #3), are factors leading to the enhancement of proteolysis in the cardiac tissue. Loss of tight control over the activity of MMP can be a driving force for the destruction of the myocardial matrix. This may result in the weakening of cardiac activity in HM conditions.

It is known that a long-term increase in the concentration of blood homocysteine can provoke the development of systemic inflammation [4, 12]. Among the serious consequences of inflammation is an impairment of proteolysis, partly due to an increase in the levels of MMPs, an expression of which is controlled by some inflammatory cytokines. The induction of the synthesis of most MMPs is mediated by pro-inflammatory cytokines such as IL-1, IL-6, TNFα, while other cytokines such as IL-4 or IL-10 have been shown to inhibit the expression of MMP genes. Thus, the revealed increase in the level of cytokines, especially IL-6 and TNFα, in the heart of HM rats may be the cause of an increase in the levels of MMPs. It should be noted that the increase in the level of MMPs can be explained not only by the influence of cytokines but also by a disturbance of oxidative balance. It was found that the metabolism of homocysteine is associated with the formation of reactive oxygen species. These metabolites can directly activate pro-MMPs, as well as activate key transcription factors that affect the expression of MMPs. Obtained data revealed an increase in the level of cytokines in the control animals with age. This may indicate a predisposition of old animals to the development of inflammation in the heart.

## CONCLUSION

1. An increase in the total proteolytic activity was observed, which was more pronounced in older animals with HM.
2. The pathogenesis of HM is associated with an increase in the level of MMP-1, -2, -3, -8 in the heart of young animals, MMP-1, -2 in the heart of adult animals, and MMP-1, -2, -8 in the heart of old animals. An increase in the percentage of metal-dependent enzymes was found, especially in the group of old rats with HM.
3. The state of mild inflammation, evidenced by the increased level of some pro-inflammatory cytokines, was revealed in the heart of young and old animals with HM.

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ORIGINAL ARTICLE

# IMPACT OF POLYCYSTIC OVARIAN SYNDROME ON GENERAL HEALTH RELATED-QUALITY OF LIFE AMONG A SAMPLE AT "MATERNITY AND CHILDREN TEACHING HOSPITAL" IN DIWANIYAH CITY-IRAQ

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## ABSTRACT

**The aim**: This study amid to identify and studying the most properties of general health related-quality of life (GHR-QoL) of polycystic ovarian syndrome (PCOS) patients, and to find out relationships among redistribution of an overall evaluation quality of life using the general scale of WHO QoL–BERF questionnaire.

**Materials and methods:** A descriptive study of patients with PCOS conducted, and it was starting the data collection from 6th December 2020up to 10thMay 2021from the "Maternity and Children Teaching Hospital" in Diwaniyah city –Iraq". Convenient sampling method of (100) patients who visited to that hospital during the data collection period had been selected.

**Results:** The findings of the study indicated that (GHR-QoL) for the studied patients are assigned that the observing responses were at moderate response generally, and they are accounted for the contents of studied questionnaire (Physical, Psychological, Social, and Environmental) main domains respectively. As well as, results shows that an overall of the (GHR-QoL) redistribution (under/upper) a cutoff point regarding percentile global mean of score (PGMS) that (SDCv.) has reported weak relationships with no significant at P>0.05.

**Conclusions**: There were weak relationships between GHR-QoL and (SDCv.), and it could be concluded that studied questionnaire could be taken a broad view on studied population even though differences with their (SDCv.) indeed.

**KEY WORDS:** Polycystic Ovarian Syndrome, PCOSQ, General Health-Related Quality of Life (GHR-QoL), WHO QoL–BERF Questionnaire

## INTRODUCTION

Topic-quality of life with reference to polycystic ovarian syndrome (PCOS) unfolds two fundamental concepts: (PCOS) which has a standard definition and quality of life which must be properly conceptualized and defined after a proper understanding of the meaning of life itself, and (PCOS) is a hormonal confusion that is prevailing in females of reproductive age with signs, symptoms that significantly decreasing self-esteem and having impact on health related quality of life (HR-QoL), [1-4]. Consequently, several investigations conducted over the world have shown associations among HR-QoL and the presence of PCOS. Women with PCOS may be at a higher risk of low HR-QoL. However, several of the previous studies have focused on series of women with PCOS or evaluated the effect of an intervention (life style or medical treatments). Therefore, Interpretation of such studies presents a significant challenge due to the varying sample sizes and tools that assess HR-QoL, heterogeneity between study groups, and different variables that have a significant impact such as age, body mass index, educational level, occupational activity, and differences in geographic areas and different races among women PCOS in HR-QoL [2]. This study are focused on how PCOS has a detrimental effect on GHR-QoL, using the general scale of WHO QoL–BERF questionnaire in compact form in order to show how this conditions affects general HR-QoL, and how the disease presents differently across studied patients among a sample at "General Women and Children Hospital" in Diwaniyah Governorate through evaluating relationships among general scales and some related variables of studied patients, such as: (Age Groups, Marital Status, Education, Education, Residency, and Socio-Economic Status).

## THE AIM

1. To identify and study most properties of General Health-Related–Quality of Life (GHR-QoL) instruments of patient's women with (PCOS).
2. To find out the relationships among the general scales evaluation (GHR-QoL) in patients with (PCOS) and some related variables of patients: (Age Groups, Marital Status, Education, Education, Residency, and Socio-Economic Status).

## MATERIALS AND METHODS

### SETTING OF THE STUDY

A cross sectional design (descriptive study) for patients with (PCOS) which were conducted starting data collection from 6th of December 2020 and 10th May 2021.

### THE SAMPLE OF THE STUDY

This study was conducted on a convenient sample of 100 adults' patients with (PCOS), who have been diagnosed and treated by "Maternity and Children teaching Hospital" in Diwaniyah city, Iraq.

### STEPS OF THE STUDY

For evaluate adults women's patients with (PCOS), appropriate instrument are selection for study of subjects, namely, (GHR-QoL), questionnaires format regarding "Health World Health Organization – WHO QoL-BERF", which consists 26 items formed four main domains, "Physical, Psychological, Social, and Environment", as well as two questions for rating and satisfying patients of QoL.

### PILOT STUDY

Reliability coefficients of the pilot study, shows that intra examiner (test & retest) equal to $[0.92_{(22:260)}]$, and inter examiners recorded highly and adequate outcomes equal to $[0.88_{(31:260)}]$, through using Al-Naqeeb Formula [3]

### STATISTICAL METHODS

Statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS), ver. 22.0: it has included on (Frequencies, and Percentages) and descriptive statistical methods such that: "Mean of score (MS), Standard Deviation (SD), Relative Sufficiency (RS%), Percentile Grand/ or Global Mean of Score (PGMS), and Pooled Standard Deviation(PSD%)", in addition to that, scoring scales concerning five ordinal scales of integer numbers of sampling zero, for the general HR-QoL. Reassessment scoring scales for tri dichotomous random variable, for scoring five ordinal scales are given by following intervals: [(20.00 – 46.66) for Low (L); (46.67 – 73.33) for Moderate (M); and (73.34 – 100) for High (H)], as well as score of percentile mean of score are given by following intervals: [(00.00 – 33.33) for Low (L); (33.34 – 66.66) for Moderate (M); and (66.67 – 100) for High (H)], "Contingency Coefficients CC" test: are estimated for association tables to find out the cause's correlation ships.

## RESULTS

Table (I) shows distribution of studied "Socio-Demographical Characteristics variables-SDCv", and "Body mass index -BMI", included the observed frequencies, and cumulative percents.

**Table I.** Distribution of the studied sample according to (SDCv) Observed Frequencies and Cumulative Percent's

| SDCv. | Classes | No. | Cum. % |
|---|---|---|---|
| Age Groups (yrs.) | < 20 | 33 | 33 |
| | 20 _ 24 | 19 | 52 |
| | 25 _ 29 | 22 | 74 |
| | 30 _ 34 | 14 | 88 |
| | > 35 | 12 | 100 |
| | Mean ± SD | 24.67 ± 7.63 | |
| Marital State | Single | 38 | 38 |
| | Married | 59 | 97 |
| | Divorced | 3 | 100 |
| Education levels | Illiterate | 6 | 6 |
| | Read & Write | 16 | 22 |
| | Primary | 34 | 56 |
| | Intermediate | 9 | 65 |
| | Secondary | 16 | 81 |
| | College & More | 19 | 100 |
| Job of patients | High professional & managerial jobs | 3 | 3 |
| | Lower professionals, skilled and semiskilled | 13 | 16 |
| | Unskilled workers | 84 | 100 |
| Residency | Urban | 88 | 88 |
| | Rural | 12 | 100 |
| Socio-Economic Status | < 60 (Low) | 55 | 55 |
| | 60 - 80 (Moderate) | 35 | 90 |
| | > 80 (High) | 10 | 100 |
| BMI | Under weight | 3 | 3 |
| | Normal weight | 24 | 27 |
| | Overweight | 36 | 63 |
| | Obese | 37 | 100 |
| | Total | 100 | - |
| | Mean ± SD | 28.58 ± 5.86 | |

Age groups shows that three quarters of less than 30 yrs. old of the studied sample are recorded at the 1st, 2nd, and 3rd age groups, and were accounted 74%, as well as mean value and standard deviation are estimated by 24.67 yrs., and 7.63 yrs. respectively, as well as 59% of studied sample were married. More than half of studied sample were recorded their educational levels at primary school or less, and were accounted 56%, and most of them has unskilled worker, and were accounted (84%), urban residency are accounted 88% among studied sample, vast majority of "Socio-Economic Status" had recorded at low, and moderate levels, and they are accounted 90.0%. Finally, results shows observed frequencies, and cumulative percents of "Body mass index -BMI", observed vast majority of studied sample had registering overweight and obesity outcome,

**Table II.** Distribution of the studied sample according to some related General Information with comparisons significant (N=100)

| Risk Factors | Response | No. | Cum. % | C.S. (*) P-value |
|---|---|---|---|---|
| Family History of PCOS | No | 51 | 51 | P=0.920 NS |
| | Yes | 49 | 100 | |
| Thinning Hair | No | 19 | 19 | P=0.000 HS |
| | Yes | 81 | 100 | |
| Hair loss from the head | No | 100 | 100 | P=0.000 HS |
| | Yes | 0 | 100 | |
| Are you under treatment now? | No | 68 | 68 | P=0.000 HS |
| | Yes | 32 | 100 | |
| Weight Gain after Menarche | No | 36 | 36 | P=0.000 HS |
| | Yes | 64 | 100 | |
| Age at Menarche | 9-10 | 21 | 21 | $\chi^2$= 29.76 P=0.000 (HS) |
| | 11-12 | 27 | 48 | |
| | 13-14 | 45 | 93 | |
| | 15-16 | 7 | 100 | |
| Menstrual cycle | Regular | 17 | 17 | P=0.000 HS |
| | Irregular | 83 | 100 | |
| Duration of menstrual cycle Per Days | No Menstrual cycle | 6 | 6 | $\chi^2$= 41.44 P=0.000 HS |
| | < 5 | 26 | 32 | |
| | 5-7 | 50 | 82 | |
| | > 7 | 18 | 100 | |
| Increasing of (Androgen Hormone) | No | 64 | 64 | P=0.000 HS |
| | Yes | 36 | 100 | |
| Regular Exercise | No | 75 | 75 | P=0.000 HS |
| | Yes | 25 | 100 | |
| Are you Diabetic | No | 98 | 98 | P=0.000 HS |
| | Yes | 2 | 100 | |
| Abnormal uterine bleeding | No | 68 | 68 | P=0.000 HS |
| | Yes | 32 | 100 | |
| Eating Disorders | No | 51 | 51 | P=0.920 HS |
| | Yes | 49 | 100 | |
| Sleep Apnea | No | 27 | 27 | P=0.000 HS |
| | Yes | 73 | 100 | |
| High Blood Pressure | No | 33 | 33 | P=0.000 HS |
| | Yes | 67 | 100 | |
| Abnormal cholesterol or triglyceride levels | No | 83 | 83 | P=0.000 HS |
| | Yes | 17 | 100 | |

*HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on One-Sample Chi-Square test, and Binomial test

and they are accounted 73.0%. Table (II) shows observed frequencies, and cumulative percents of "Risk Factors", with comparisons significant.

Regarding to subject "Family History of PCOS", half of studied respondents having a family history, and they are accounted 49%, most of studied patients has a "Thinning Hair", and they are accounted 81%, no one of studied patients having hair loss from head, 32% of studied patients are under treatment, two third of studied patients has a "Weight Gain after Menarche", and they are accounted 64%, about half of studied respondents 52% had a menarche age between (13–16) yrs, and 83% had irregular menstrual cycle, and only 6% among studied patients has no menstrual cycle, and half of them duration of menstrual cycle taken (5–7) days, and 18% of the leftover taken more than 7 days, 36% of studied patients are recorded increasing of (Androgen Hormone), 75% of studied patients hadn't regular exercise. Only 2% among studied patients has a diabetic disease, 32% among them has abnormal uterine bleeding, 49% among them has eating disorder, 73% among them has sleep apnea, 67% among them has high blood pressure, and 17% among them has abnormal cholesterol or triglyceride levels.

## GENERAL HEALTH RELATED QOL

Regarding subjects GHR-QoL, table (III) shows summary statistics of initial evaluation, such as, mean of score, standard deviation, and relative sufficiency's, as well as different responses levels of evaluating GHR-QoL through transform scoring scales by three differentiate categories, such that (Low, Moderate, and High). General quality of life for the studied patients assigned observed responses are moderate mostly, and they are accounted 24 (92.31%).

## DISTRIBUTION OF QUESTIONNAIRE'S DOMAINS (GENERAL HR-QOL)

Regarding subjects of main domains, table (IV) shows summary statistics, such that, percentile score, and standard deviation, as well as different responding levels of evaluating of main domains for general HR-QoL through percentile transforming scoring scales by three differentiated categories' levels, such that (Low, Moderate, and High) of WHO QoL – BERF questionnaire, which consists of (Physical, Psychological, Social, and Environment) main domains.

## PGMS: PERCENTILE GRAND MEAN OF SCORE; PSD: PERCENTILE STANDARD DEVIATION

To find out relationships amongst redistribution of overall evaluation through (under/upper) cutoff point of percentile grand mean of score concerning of [General, and Specific–HRQoL] of PCOS's patients and their [Socio-Demographical Characteristics] variables, such that: (Age Groups, Marital State, Education, Occupation, Residency, and Socio-Economic Status), as illustrated in table (V) which consists a contingency coefficients and test hypotheses, which says that meaningless relationships are accounted between redistribution of preceding variables.

Results shows that weak relationships were accounted amongst redistribution of overall general HR-QoL of

**Table III.** Summary Statistics of patients responding concerning General Quality of Life's items (N=100)

| General HRQoL | MS | SD | RS | Ev. |
|---|---|---|---|---|
| How would you rate your QOL? | 2.90 | 0.97 | 58.0 | M |
| How satisfied are you with your health? | 2.86 | 1.04 | 57.2 | M |
| To what extent do you feel that physical pain prevents you from doing what you need to do? | 2.92 | 1.07 | 58.4 | M |
| How much you need any medical treatment to function in your daily life? | 2.58 | 1.32 | 51.6 | M |
| How much do you enjoy life? | 2.91 | 1.12 | 58.2 | M |
| To what extent do you feel your life to be meaningful? | 3.00 | 1.09 | 60.0 | M |
| How well are you able to concentrate? | 3.21 | 1.08 | 64.2 | M |
| How safe do you feel in your daily life? | 3.12 | 1.14 | 62.4 | M |
| Ho healthy is your physical environment? | 3.37 | 0.91 | 67.4 | M |
| Do you have enough energy for everyday life? | 3.02 | 1.09 | 60.4 | M |
| Are you able to accept your bodily appearance? | 3.38 | 1.33 | 67.6 | M |
| Have you enough money to meet your needs? | 3.07 | 0.86 | 61.4 | M |
| How available to you is the information that you need in your day to day life? | 3.61 | 1.19 | 72.2 | M |
| To what extent do you have opportunity for leisure activities | 3.32 | 1.27 | 66.4 | M |
| How well are you able to get around? | 3.37 | 1.32 | 67.4 | M |
| How satisfied are you with your sleep? | 3.39 | 1.05 | 67.8 | M |
| How satisfied are you with your ability to perform your daily living activities? | 3.27 | 1.02 | 65.4 | M |
| How satisfied are you with your capacity for work? | 3.51 | 0.87 | 70.2 | M |
| How satisfied are you with yourself? | 3.60 | 0.98 | 72.0 | M |
| How satisfied are you with your personal relationships? | 3.67 | 0.91 | 73.4 | H |
| How satisfied are you with your sex life? | 2.61 | 1.54 | 52.2 | M |
| How satisfied are you with support you get from your friends? | 3.42 | 1.07 | 68.4 | M |
| How satisfied are you with the conditions of your living place? | 3.32 | 1.01 | 66.4 | M |
| How satisfied are you with your access to health services? | 3.21 | 0.99 | 64.2 | M |
| How satisfied are you with your transport? | 3.32 | 1.01 | 66.4 | M |
| How often do you have negative feeling such as blue mood, despair, anxiety, depression? | 3.72 | 1.24 | 74.4 | L |

MS: Mean of Score; SD: Standard deviation according to MS.
RS%: Relative Sufficiency Assess by (L: Low; M: Moderate; H: High)
Red color items reversed measuring scale, and that revere an evaluates of using score

PCOS's patients and their (SDCv) at P>0.05, and according to that it could be conclude that studied of WHO QoL–BERF questionnaire can be amend for studied phenomena on the target population rather than differences among those who had PCOS's patient's socio-demographical characteristics variables.

## DISCUSSION

To best of our knowledge, this study is the first attempt to investigate evaluation of GHR-QoL regarding patients with PCOS in Iraq, since it came with comprehensive coverage to include all areas of study subjects, regarding general components. By reviewing the results of the preliminary data, specifically the SDCv. of patients, it has been noticed that most of them are focusing at differentiated levels, such as age below (30) yrs., two third were married, more than half of studied sample were recorded their educational levels at the primary school or less, most of them has unskilled worker, and finally urban

residency, and that are registered an agreement with the finding of the others studies, such as carried out in India titled Impact of PCOS on quality of life concerning women in correlation to age, basal metabolic index, education and marriage, which stated that majority of the PCOS age patients are recorded 78% [4], as well as 59% of subjects were married, and this finding agreed with another study in Iran [5], concerning the level of education, most of the studied sample having primary school or less, this result in agreement with [6] in Iran. Relative to employment, the results indicating that more than half of the study sample is unskilled worker and housewives, this finding was similar to study done by [7], in addition, its agreement with the study done by [8]. Regarding residency highest percentage of the current study sample are living in urban area, and that findings is similar and in agreement with [9] study. Finally, Socio-Economic Status-SES with comparison significant, and they are accounted through applying WHO instrument, and Al-Naqeeb correction, which consists of several components such that, occupation, education levels, crowding index (no.

**Table IV.** Summary Statistics of Percentile Score General QoL main domains for the studied patients

| Main Domains | No. | PGMS | PSD | Evaluated |
|---|---|---|---|---|
| Physical Domain | 100 | 57.36 | 13.44 | Moderate |
| Psychological Domain | 100 | 51.58 | 15.66 | Moderate |
| Social Domain | 100 | 55.83 | 18.93 | Moderate |
| Environment Domain | 100 | 57.31 | 11.77 | Moderate |
| General HR-QoL | 100 | 55.52 | 9.82 | Moderate |

**Table V.** Relationships among General HR-QoL concerning PCOS Patients in light of Socio-Demographical Characteristics variables

| SDCv. | General – QoL | |
|---|---|---|
| | C.C. | P-value[(*)] |
| Age Groups | 0.109 | 0.879 (NS) |
| Marital State | 0.088 | 0.675 (NS) |
| Education | 0.114 | 0.934 (NS) |
| Occupation | 0.189 | 0.157 (NS) |
| Residency | 0.017 | 0.868 (NS) |
| Socio-Economic Status | 0.172 | 0.217 (NS) |

*NS: No Sig. at P>0.05; Statistical hypothesis based on Contingency's Coefficient test

of households, and no. of rooms), and a particular property (house ownership, possession of a car, available of represented by the preceding contents (Low, Moderate, and High). The current study has recorded high percent 90.0% concerning on the low, and moderate levels, and that is in agreement with a study done in Wuhan University [10], since it was accounted high percent of studied subjects in low, and moderate levels, and they are accounted 90.3% and it is too highly corresponding to the current study indeed. BMI for the current study equal to three quarters of the studied sample reported an overweight, and obesity levels, and they are accounted 73.0%, with mean and standard deviation (28.58 and 5,86) respectively, and that is in agreement with the study done by Dogan in Turkey, which reported mean and standard deviation 30.7 and 4.88, respectively [11]. By reviewing the results of "General Information", specifically the (Risk Factors) associated with the PCOS patients, it has been noticed that most of them are focusing at differentiated levels, such as Family History which are accounted 49%, most of patients having Thinning Hair and they are accounted 81%, no one of studied patients having hair loss from head, 32% of studied patients are under treatment, two third of studied patients has a "Weight Gain after Menarche", and they are accounted 64%, about half of studied respondents 52% had a menarche age between (13–16) yrs and 83% had irregular menstrual cycle, and only 6% among studied patients has no menstrual cycle, and half of them duration of menstrual cycle taken (5–7) days, and 18% of the leftover taken more than 7 days, 36% of studied patients are recorded increasing of (Androgen Hormone), 75% of studied patients hadn't regular exercise. Studied patients were diagnosed with irregular menstrual cycle 83%, which represent second highest percentage of

other included and non-convergence done by Ghodsi in Iran 38.5% [12], also sleep apnea, which is similar by Kahal done in UK, 60% [13], high blood pressure which is similar by Joham in Australia 55.5%, study a cross-sectional analysis [14], followed by family history agreed done by Jungari in India 50% [15], Androgen hormone approach done by Ghodi in Bangladeshi 33.6% [12]. Main domains concerning (General HR-QoL), Physical Domain" as mentioned by PGMS, and PSD 57.36 and 13.44, respectively was evaluated on moderate. Domain: 1 (Physical Domain), which is content the activities of daily living, dependency on medicinal substances and medical aids, energy and fatigue, mobility, pain and discomfort, sleep and rest, work capacity as this field shows individual's ability to engage in general physical activities with PCOS, and studies confirmed the importance of this area, as Rzońca in Poland 2018, agreed with the current study with PGMS, and PSD 53.88 and11.87, respectively. Psychological domain on PCOS patients, where the current study showed a moderate response with PGMS, and PSD 51.56 and 15.66, respectively, domain: 2 (Psychological Domain) was maximally affected domain which involves a self-esteem, negative and positive feelings, bodily image and appearance, spirituality, religion, personal beliefs, thinking, learning, memory and concentration agreed the current study with Prathap in India with PGMS, and PSD 68.80 and 12.87, respectively, [16]. With reference to social main domain General HR-QoL contains three items concerned with personal relationships, sexual life and getting support from relative persons, the present study estimated moderate responses with PGMS, and PSD 55.83 and 18.93, respectively, which means that PCOS patients have problems regarding social issues in moderate level, this finding in similar with a study done in India by Prathap, which is a cross-sectional design study. The health quality of life regarding preceding domain conducted in Portugal was not alike of this study, since it recorded high evaluated of PGMS, and PSD 80.93 and 18.14, respectively, [17]. Regarding environmental main domain General HR-QoL where the current study showed moderate evaluation by PGMS, and PSD (57.31and 11.77) respectively, and that is agreed with study done by Aduloju in Nigerian with PGMS 58.27 [18]. Relationships related to General HR-QoL of PCOS patients and their SDCv, such that (Age Groups, Marital State, Education, Occupation, Residency, and Socio-Economic Status), results shows weak relationships.

## CONCLUSIONS

This study showed that patients with (PCOS) having go down concerning general HR-QoL, since most of studied items regarding WHO QoL–BERF questionnaire, since are accounted moderate evaluation, and this was achieved to a similar degree across all domains of the questionnaire. In light of this, the importance of studying health status evaluation for quality of life of patients with the syndrome is confirmed, according to the aforementioned questionnaire, due to its high ability to detect the reservoirs of the effects caused by the (PCOS). As well as, weak relationships has been accounted by redistribution of overall evaluations concerning general HR-QoL independently for the studied patients with PCOS and

their differentiated SDCv and according to that, it could be conclude that studied of WHO QoL–BERF questionnaire for general HR-QoL, for women with (PCOS) could be amending for studied phenomena on the sampling population rather than differences among their SDCv.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

# PECULIARITIES OF THYROID STATUS OF PRESCHOOL CHILDREN WITH ACUTE BRONCHITIS

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**ABSTRACT**

**The aim:** The aim of this study was to determine the characteristics of thyroid status (thyroid hormone, total and free fractions of triiodothyronine and thyroxine, reversible triiodothyronine, antibodies to thyroperoxidase) of preschool children with acute bronchitis.

**Materials and methods:** We examined 135 preschool children (from 3 to 6 years old) with acute bronchitis (main group) and 28 apparently healthy subjects who were in the control group. It used clinical-anamnestic, laboratory and instrumental research methods. Evaluation of the course of acute bronchitis was carried out in the acute period of the disease. All results were statistically processed using the SPSS 26 package.

**Results:** In 33 % of patients with acute bronchitis there are subclinical abnormalities of thyroid hormones, which manifest themselves in the form of euthyroid sick syndrome. Namely, we found an increased concentration of reversible triiodothyronine in the serum, as well as a decrease in total triiodothyronine and its free fraction.

**Conclusions:** In patients with acute bronchitis in almost every third case there are functional shifts in hormonal status, which are manifested in the form of the first variant of the euthyroid sick syndrome.

   **KEY WORDS:** acute bronchitis, children, euthyroid sick syndrome, triiodothyronine, thyroxine

## INTRODUCTION

An analysis of scientific publications shows that over the last century, acute infectious diseases of the respiratory system among children of all ages occupy a leading place and as a result form an important medical, social, economic and scientific problem. Respiratory tract diseases account for the vast majority, up to 90 %, of reported cases among children. According to the WHO, every year 50 % of children under the age of 5 suffer from acute respiratory infections, and from 5 to 12 years – 30 %. Acute infections of the respiratory system are 7 – 7.5 times higher than the incidence of all other infections and 1.5 – 3 times higher than in adults [1].

Acute bronchitis with its various clinical forms occupies a significant share in the structure of nonspecific acute infectious diseases of the respiratory system of children. Thus, the incidence of this nosology averages about 100 per 1,000 children [2]. Acute bronchitis is one of the five reasons for outpatient treatment by a pediatrician [3]. Among the hospitalized patients in children's infectious wards with respiratory lesions in every 2 – 4 cases in the history of the disease there is a diagnosis of acute bronchitis [4].

Acute pathological processes accompanied by inflammation, including acute bronchitis, in the course of stressful changes involve the hypothalamic-pituitary-thyroid system. Therefore, the state of thyroid status depends on the resistance and adaptive stability of the organism, which can deter-mine the features of acute bronchitis and the consequences for the patient [5, 6, 7]. In particular, such an adaptive state as euthyroid sick syndrome (ESS) is developing, which is manifested by various combinations of changes in thyroid hormone concentrations at the subclinical level [8-10]. Most often there is the first variant of this pathology: ESS-1 or "low T3 syndrome" [8, 11]. The study of this condition will better understand the interdependence of hormonal and immune systems, their response to acute inflammatory process of the respiratory tract in children and the stress that accompanies it, as well as further use the data to predict the course of acute bronchitis and improve treatment approaches.

After analyzing the available sources of information, we found that the state of the thyroid system of preschool children with acute bronchitis remains unresolved. Therefore, we see the relevance in the study of this problem, which will improve the early diagnosis of the features of acute bronchitis and predict the consequences for the patient.

## THE AIM

The aim of this study was to determine the characteristics of thyroid status (thyroid hormone, total and free fractions of triiodothyronine and thyroxine, reversible triiodothyronine, antibodies to thyroperoxidase) of preschool children with acute bronchitis.

Minimum - minimum meaning

Maximum – maximum meaning

Mean – middle meaning
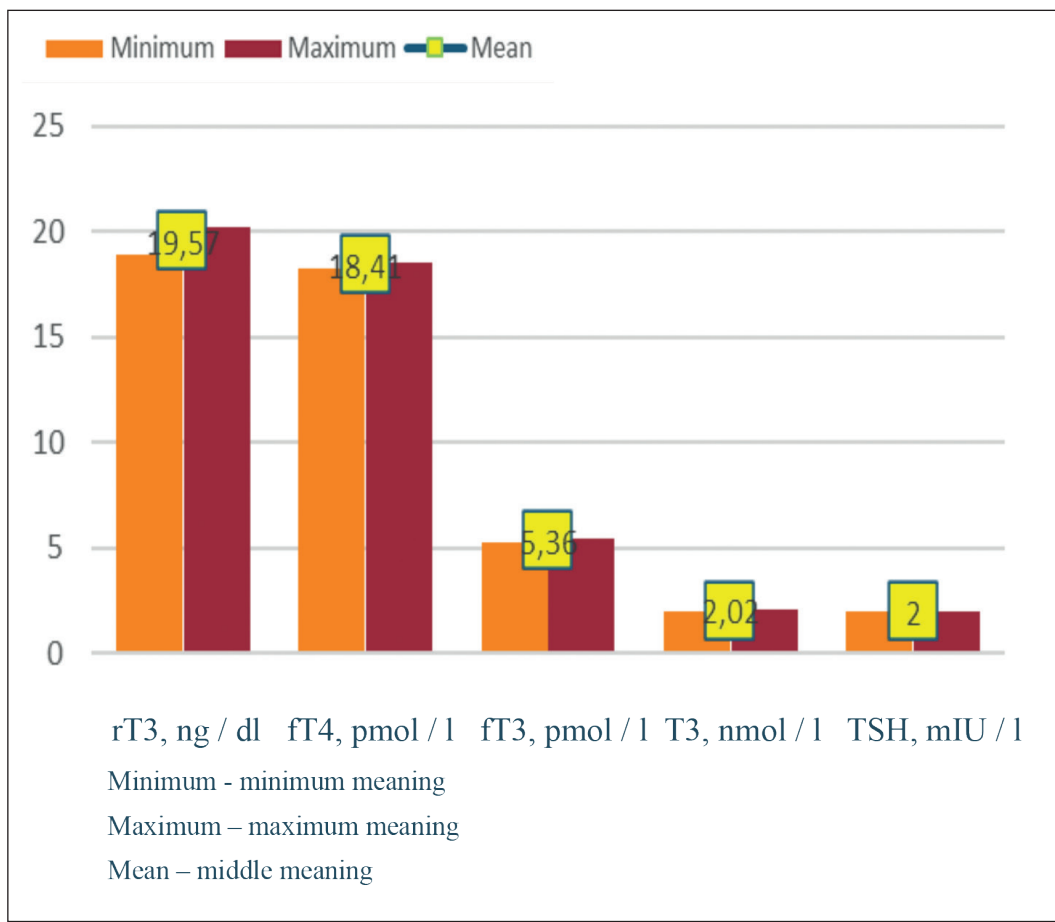
**Fig. 1.** Levels of thyroid hormones in the serum of the main group in the acute period of the disease

**Table I.** Condition of thyroid hormones in the serum of children with acute bronchitis (main group) in the acute phase of the disease

| Hormones | N | Minimum | Maximum | Mean | |
| --- | --- | --- | --- | --- | --- |
| | Statistic | Statistic | Statistic | Statistic | Std. Error |
| T4, nmol / l | 135 | 118,60 | 150,40 | 122,7178 | 0,41377 |
| rT3, ng / dl | 135 | 9,40 | 37,60 | 19,5733 | 0,68367 |
| fT4, pmol / l | 135 | 6,00 | 21,70 | 18,4126 | 0,15247 |
| fT3, pmol / l | 134 | 3,50 | 6,70 | 5,3597 | 0,10433 |
| T3, nmol / l | 135 | 1,01 | 2,74 | 2,0225 | 0,05069 |
| TSH, mIU / l | 135 | 1,13 | 3,58 | 1,9956 | 0,04281 |

N – number

Statistic – meaning

Minimum – minimum meaning

Maximum – maximum meaning

Mean – middle meaning

Std. Error – standart error

## MATERIALS AND METHODS

We examined 135 preschool children (3 to 6 years old) with acute bronchitis (main group) who were treated in the infectious diseases departments of the Municipal Non-Profit Enterprise "Children's Clinical Hospital of St. Zinaida" Sumy City Council and 28 healthy children (control group) who were under supervision pediatrician in the Municipal Non-Profit Enterprise "Center for Primary Health Care № 2" Sumy City Council. The first group consisted of 92 children with acute bronchitis, the second group included 43 patients with acute bronchitis with signs of ESS.

The examination of the children included in the study was agreed with their parents (informed consent) and met the requirements of bioethics, as evidenced by the protocol of the Commission on Biomedical Ethics of the Municipal Non-Profit "Children's Clinical Hospital St. Zinaida" Sumy City Council.

Clinical and anamnestic assessments were used to comprehensively assess the patient's thyroid status (collection of complaints, anamnesis of disease and life, physical examination), laboratory (determination by solid-phase

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**Table II.** The state of thyroid hormones in children with acute bronchitis with signs of ESS and without them (main group) in the acute phase and control groups

| | Groups | TSH, mIU / l | T3, nmol / l | fT3, pmol / l | rT3, ng / dl | T4, nmol / l | fT4, pmol / l |
|---|---|---|---|---|---|---|---|
| I | Mean | 2,0059 | 2,4134 | 6,1620 | 14,5478 | 122,6707 | 18,5652 |
| | N | 92 | 92 | 92 | 92 | 92 | 92 |
| | Std. Error of Mean | 0,04940 | 0,01581 | 0,02418 | 0,23458 | 0,52575 | 0,14839 |
| II | Mean | 1,9735 | 1,1863 | 3,6024 | 30,3256 | 122,8186 | 18,0860 |
| | N | 43 | 43 | 42 | 43 | 43 | 43 |
| | Std. Error of Mean | 0,08383 | 0,01195 | 0,01050 | 0,62251 | 0,65813 | 0,35646 |
| Control group | Mean | 1,8857 | 2,3732 | 6,2107 | 15,8679 | 121,8571 | 19,0357 |
| | N | 28 | 28 | 28 | 28 | 28 | 28 |
| | Std. Error of Mean | 0,04437 | 0,02619 | 0,03014 | 0,16011 | 0,60477 | 0,32060 |
| Total | Mean | 1,9767 | 2,0828 | 5,5068 | 18,9368 | 122,5699 | 18,5196 |
| | N | 163 | 163 | 162 | 163 | 163 | 163 |
| | Std. Error of Mean | 0,03637 | 0,04345 | 0,09004 | 0,57706 | 0,35834 | 0,13860 |

Mean – middle meaning
N – number
Std. Error of Mean – standart error of middle meaning
Total – total

**Table III.** Dispersion analysis of differences in the state of hormones of children with acute bronchitis with signs of ESS and without them (main group) in the acute phase and control groups

| | Hormones | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|---|
| TSH, mIU / l | Between Groups | (Combined) | 0,031 | 1 | 0,031 | 0,123 | 0,726 |
| | Within Groups | | 33,123 | 133 | 0,249 | | |
| | | | 33,154 | 134 | | | |
| T3, nmol / l | Between Groups | (Combined) | 44,124 | 1 | 44,124 | 2497,172 | 0,000 |
| | Within Groups | | 2,350 | 133 | 0,018 | | |
| | Total | | 46,474 | 134 | | | |
| fT3, pmol / l | Between Groups | (Combined) | 188,916 | 1 | 188,916 | 4902,456 | 0,000 |
| | Within Groups | | 5,087 | 132 | 0,039 | | |
| | Total | | 194,002 | 133 | | | |
| rT3, ng / dl | Between Groups | (Combined) | 7294,793 | 1 | 7294,793 | 835,988 | 0,000 |
| | Within Groups | | 1160,551 | 133 | 8,726 | | |
| | Total | | 8455,344 | 134 | | | |
| T4, nmol / l | Between Groups | (Combined) | 0,641 | 1 | 0,641 | 0,028 | 0,868 |
| | Within Groups | | 3096,416 | 133 | 23,281 | | |
| | Total | | 3097,057 | 134 | | | |
| fT4, pmol / l | Between Groups | (Combined) | 6,728 | 1 | 6,728 | 2,162 | 0,144 |
| | Within Groups | | 413,820 | 133 | 3,111 | | |
| | Total | | 420,549 | 134 | | | |

Sum of Squares – sum of squares
Df – definition degrees of freedom
Mean Square – mean values of squares
F - the value of Fisher's calculation criterion
Sig. – significant - p-value (materiality)
Between Groups | (Combined) – intergroup (combined)
Within Groups – in the middle of groups (average of groups)
Total - total

enzyme-linked immunosorbent assay) of serum hormones such as thyroid-stimulating hormone (TSH), total and free (T3 and fT3) and thyroxine (T4 and fT4), reversible triiodothyronine (rT3), antibodies to thyroperoxidase), as well as instrumental research methods (chest radiography, ultrasound examination of the thyroid gland).

In children of the main group, the diagnosis of acute bronchitis was verified on the basis of complaints of children and their parents, anamnesis, objective symptoms, results of laboratory and instrumental research methods according to the clinical protocol on medical care for children in "Pediatric Pulmonology" from 13.01.2005 № 18 with changes and additions made by the order of the Ministry of Health of Ukraine from July 16, 2014 № 499 (Unified clinical protocol of primary care for adults and children "Acute respiratory infections"), Adapted clinical guidelines based on evidence, " Influenza and acute respiratory infections ", 2014. Assessment of acute bronchitis, including the use of laboratory and instrumental methods, was carried out in the acute period of the disease (1 – 2 days of hospitalization).

In addition, we studied children in the main group of ESS, namely the first option, known as "low T3 syndrome". This is an adaptive state in which non-thyroid diseases with euthyroidism cause subclinical changes in serum thyroid hormone levels: decreased T3, fT3 and increased rT3.

All results were statistically processed using the SPSS 26 package. Descriptive statistics and comparisons of mean values were used to characterize the course of acute bronchitis in preschool children. Differences between groups were confirmed or refuted by analysis of variance for quantitative characteristics and the chi-square criterion, for features of nominal or rank scale.

## RESULTS

Dynamic determination of hormonal status was performed on all 135 preschool patients of the main group and 28 children of the control group. Clinically pronounced hypo- or hyperthyroidism was not detected in the examined children. With the help of ultrasound examination of the thyroid gland was ruled out the presence of organic pathology of these organs. The diagnosis of pneumonia was refuted in doubtful clinical cases after X-ray examination of the chest. Determination of the concentration of antibodies to thyroperoxidase made it possible to exclude the autoimmune etiology of changes in thyroid hormones.

The concentrations of hormones in the serum of the control group corresponded to the age norm. Their average values were: T3 – $2.37 \pm 0.03$ nmol / l, fT3 – $6.21 \pm 0.03$ pmol / l, T4 – $121.86 \pm 0.60$ nmol / l, fT4 – $19.04 \pm 0.32$ pmol / l, TSH – $1.89 \pm 0.04$ mIU / l and rT3 – $15.87 \pm 0.16$ ng / dl.

Compared with the indicators of the control group, the children of the main group had significant deviations and variance in the values of thyroid hormones. In general, in the acute period of the disease, the level of T3 in the serum varied from 1.01 to 2.74 nmol / l, in fT3 – from 3.5 to 6.7 pmol / l, T4 – from 118.6 to 150.4 nmol / l , fT4 – from 3.5 to 6.7 pmol / l, TSH – from 1.13 to 3.58 mIU / l and rT3 – from 9.4 to 37.6 ng / dl (table I).

Average values of concentrations of hormones of the main group of the population: T3 – $2.02 \pm 0.0.5$ nmol / l, fT3 – $5.36 \pm 0.10$ pmol / l, T4 – $122.7 \pm 0.41$ nmol / l, fT4 – $18.41 \pm 0.15$ pmol / l, TSH – $1.99 \pm 0.04$ mIU / l and rT3 – $19.57 \pm 0.68$ ng / dl (Figure 1).

Studies in children with acute bronchitis with signs of ESS and without them revealed an imbalance in changes in thyroid hormones (table II).

Analyzing the data in Tables II and III, it was found that the average level of TSH, T4 and fT4 with a probability of 95 % ($p > 0.05$) did not differ statistically for the group of children with acute bronchitis with signs of ESS, without signs of ESS and in the group control.

In contrast, the level of rT3 was significantly higher in the group of children with acute bronchitis with signs of ESS ($30.32 \pm 0.62$) ng / dl against ($14.55 \pm 0.23$) ng / dl in the group of children with acute bronchitis without signs of ESS in the control group ($15.87 \pm 0.16$) ng / dl) ($p < 0.001$), respectively). The level of T3 was significantly lower in the group of children with acute bronchitis with signs of ESS ($1.18 \pm 0.01$) nmol / l against ($2.41 \pm 0.02$) nmol / l in the group of children with acute bronchitis without signs of ESS and in the control group ($2.37 \pm 0.03$) nmol / l ($p < 0.001$), respectively; and fT3 in the group of children with acute bronchitis with signs of ESS ($3.6 \pm 0.01$) pmol / l against ($6.16 \pm 0.2$) pmol / l in the group of children with acute bronchitis without signs of ESS and in control group ($3.6 \pm 0.01$) pmol / l ($p < 0.001$). The above features of changes in the concentrations of thyroid hormones in the serum and gave us grounds for selection within the main group of children with manifestations of "low T3 syndrome".

The share of functional shifts in hormones is quite large. Almost every third patient (43 ($31.85 \pm 4.02$) %)) showed changes in ESS.

## DISCUSSION

The problems of euthyroid sick syndrome in non-thyroid diseases are widely discussed in the modern medical literature. Thus, a number of authors observed euthyroid sick syndrome in patients with COVID-19, which was significantly associated with the severity of the disease and baseline parameters [12]. Other researchers have shown that levels of free T3 can serve as a prognostic criterion for determining the severity of the disease in the early manifestations of COVID-19 [13].

It is interesting to note that low levels of T3 are common among patients who are not in critical condition. Serum T3 levels, alone or in combination with other prognostic estimates, have been shown to be a simple and valuable tool for stratification of disease risk [14]. In addition, this hormone plays an important role in regulating the immune response, inducing protection against systemic inflammation in response to endotoxemia. Moreover, the key role of T3 is assigned to inhibit the differentiation of monocytes into macrophages [15]. At

the same time, new experimental and clinical studies show the evolutionary effect of T3 on the adaptation of affected tissue in hypoxia and on the immune response and viral load in infected tissue [16]. Some authors suggest that changes in the concentration of thyroid hormones in severe disease are a protective reaction, as it prevents excessive tissue catabolism [17].

A number of authors found that T3 was significantly correlated with lymphocyte counts in patients with bacterial sepsis, and lower hormone levels were found in patients with severe lymphopenia. In patients with severe lymphopenia, COVID-19 showed significantly lower plasma concentrations of TSH, T4, fT4 and T3 compared to patients without lymphopenia. At the same time, the indicators of inflammatory markers increased significantly: interleukin-6, C-reactive protein and ferritin [18].

Researchers believe that an increase in rT3 is a predictor of both short-term and long-term mortality. A decrease in the concentration of fT3 is a contributing factor to subsequent cardiac disorders [19].

The relationship between thyroid hormones and immune cells is complex, and T3 can model various aspects of innate and adaptive immune responses through both genomic and non-genomic mechanisms. Thyroid hormones have also been shown to affect the activity of natural killer cells and the cellular immune response [20].

Studies by Mei-Xian Xu and others (2020) have shown that children with sepsis often have euthyroid syndrome, which the authors believe is associated with high levels of interleukin-6 [21]. During the experiment, other researchers found that in patients with irritable bowel syndrome, changes in thyroid hormone levels are associated with a moderate increase in proinflammatory cytokines (tumor necrosis factor-alpha, interleukin-1β, interleukin-6 and interleukin-8) and decreased anti-inflammatory cytokine. (interleukin-10) [22].

The problem of a clear distinction between low T3 syndrome and central hypothyroidism has been addressed by a number of researchers and proposed to determine the serological threshold of the ratio of fT3 to fT4 (pg / ml, ng / dl, respectively) at 2.0 [23].

It is possible that in the acute phase of the disease euthyroid sick syndrome is part of the body's favorable adaptation to reduce energy expenditure and activation of the innate immune response, which is important for recovery [24]. At the same time, in chronic disease, such a hypometabolic state leads to thyroid allostasis type 1, in which the amount of energy consumed and mobilized from reserves is less than the need for it [25]. To emphasize the compensatory-adaptive orientation, other researchers suggest a new name for this condition – the syndrome of adaptive thyroid imbalance. Speaking of classification, the authors point to both immobilizing (inhibitory) and mobilizing (activating) shifts in thyroid hormones [26].

Thus, the euthyroid sick syndrome occurs in a significant proportion of hospitalized patients and includes many changes in the axis of the hypothalamus-pituitary-thyroid gland. One of the hallmarks of the syndrome is a decrease in the concentration of thyroid hormones in the serum, which is often seen as an adaptive mechanism for energy savings. Cytokines released during the disease affect a number of genes involved in the metabolism of thyroid hormones, and therefore are considered the main determinants of euthyroid sick syndrome [27].

## CONCLUSIONS

Thus, in patients with acute bronchitis in the acute period of the disease in almost every third case there are functional shifts in hormonal status, which manifests itself in the form of "low T3 syndrome" (ESS-1). It should be noted that all changes are subclinical in nature, so no signs of hypo- or hyperthyroidism were detected. Thus, in children of the selected subgroup with signs of euthyroid sick syndrome, an increased concentration of reversible triiodothyronine in the serum, as well as a decrease in the total and free fraction of triiodothyronine. Our research has shown that, in response to inflammation, children with acute bronchitis may have a physiological adaptive state involving the hypothalamic-pituitary-thyroid system.

Prospects for further research are to expand the study of hormonal homeostasis, including the hypothalamus, pituitary gland and thyroid gland, to understand the role of thyroid hormones in children with acute bronchitis in age. In addition, it is important to link thyroid hormone levels to the function of the immune system and respiratory pathology. Further research in this area is needed, which is especially important in children with infectious diseases. This will allow a more accurate understanding of the pathogenetic processes of the disease and in the long run to optimize early diagnosis, the severity of acute bronchitis and predict the consequences for the patient.

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ORIGINAL ARTICLE

# HYGIENIC ASSESSMENT OF THE INFLUENCE OF PESTICIDES ON THE FATTY COMPOSITION OF SUNFLOWER SEED LIPIDS

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## ABSTRACT
**The aim:** Evaluation of the influence of plant protection chemicals on the fatty acid composition of sunflower seeds.

**Materials and methods:** Study of the effects of pesticides, study of the effects of pesticides on the fatty acid composition (lipid) of sunflower seed by gas-liquid chromatography was studied.

**Results:** It was found that the content of oleic and linoleic unsaturated fatty acids did not differ significantly from the control. The content of linolenic and arachidonic acids was at the level of the control group. A similar pattern was observed in relation to the content of saturated fatty acids, in particular myristic, pentadecanoic, palmitic, margaric, stearic. The total content of fatty acids (unsaturated fatty acids and saturated fatty) selected under different seed protection schemes did not differ significantly from control.

**Conclusions:** It is proved that the application of the investigated pesticides in various schemes of chemical protection of sunflower crops does not affect the nutritional and biological value of the seeds.

**KEY WORDS:** biological value of sunflower seeds, pesticides, fatty acid composition, lipids, sunflower oil

## INTRODUCTION
Today, Ukraine is one of the dominant countries in the cultivation and processing of sunflower [1, 2]. Thus, according to the State Statistics Service, Ukraine maintains its leading position on sunflower oil exports and over the past years has been the leading exporter of sunflower oil, ahead of major oil-importing countries – India, China, European Union countries such as Spain, the Netherlands, Italy, etc. [3-5].

Vegetable fats play a very important role in the human body. They perform energy function, are structural elements of cell membranes, carriers and solvents of fat-soluble vitamins, form biological regulators (factor of activation of platelets, eicosanoids, lipo- and hydroperoxides, sex and steroid hormones), carry out receptors, regulate activity of transcription factors, lipid modification of proteins (attachment of fatty acids to proteins, which provides lipid – protein and protein – protein interactions (bonds), perform regulatory function (according to cholesterol levels increase, regulate hormonal balance), have a protective function, are a source for the formation of endogenous water, are involved in the transmission of intracellular signals, and also improve the taste of food [6, 7].

Therefore, sunflower oil is a useful food, first of all, as a source of polyunsaturated fatty acids (PUFA). Thus, in the fatty acid spectrum, it contains a significant amount of linoleic acid (about 80%), which belongs to the family ω6 and oleic acid (about 10-14%), which belongs to the family ω9 and is extremely useful for the human body.

Unrefined oil is also a powerful source of phospholipids and vitamin E [7].

Thus, the issues of increasing yields, preserving the nutritional and biological value of crops, including sunflower, are very relevant

Sunflower yields depend to a large extent on the phytosanitary status of agricultural land. One of the main reasons for the decline in yield is damage to plants by diseases and pests such as phomopsis, lupus, white, gray and dry rot, malfunctioning powdery mildew, phomos, rust, larvae of pieces (drones), various types of bugs, including berry, field, alfalfa and violation of scientifically based alternation of crops in rotation, returning it to the same branches in 1-4 years [4-6].

An important component of intensive sunflower growing technology is a robust chemical protection system. Chemicals for crop protection for weed control, pest and disease control include herbicides, insecticides, fungicides and desiccants. However, the effect of pesticides on the lipid composition of sunflower seeds is still unknown.

Thus, studying the possible (both quantitative and qualitative) effects of pesticides on the fatty acid composition of sunflower seeds is an important part of assessing the effects of pesticides on the biological value of plants.

## THE AIM
To study the evaluation of the influence of plant protection chemicals on the fatty acid composition of sunflower seeds.

**Table I.** Fatty Acid Spectrum of Unsaturated Sunflower Seed Fatty Acids Grown Using Different Plant Chemical Protection Schemes (in %)

| № of sample | Fatty acids | | | |
|---|---|---|---|---|
| | oleic | linoleum | linolenic | arachidonic |
| 1. | 17,9 | 67,8 | 0,3 | 0,3 |
| 2. | 14,5 | 77,4 | 0,2 | 0,2 |
| 3. | 9,5 | 81,6 | 0,2 | 0,2 |
| 4. | 10,4 | 80,5 | 0,2 | 0,2 |
| 5. | 9,3 | 81,3 | 0,2 | 0,2 |
| 6. | 11,2 | 79,6 | 0,1 | 0,1 |
| 7. | 12,0 | 78,7 | 0,2 | 0,2 |
| 8. | 13,8 | 77,4 | 0,2 | 0,3 |
| 9. | 11,1 | 80,4 | 0,2 | 0,2 |
| 10. | 9,6 | 82,6 | 0,2 | 0,2 |
| 11. | 10,7 | 81,9 | 0,1 | 0,2 |
| 12. | 10,0 | 82,0 | 0,1 | 0,1 |
| 13. | 12,0 | 81,3 | 0,2 | 0,2 |
| 13. | 11,0 | 81,0 | 0,2 | 0,2 |
| 15 | 11,8 | 79,7 | 0,2 | 0,2 |
| 16 | 12,7 | 79,4 | 0,2 | 0,3 |
| 17 | 9,4 | 83,3 | 0,2 | 0,1 |
| 18. c' | 11,1 | 80,0 | 0,2 | 0,2 |
| 19.c' | 11,0 | 82,0 | 0,2 | 0,2 |
| 20.c' | 10,6 | 82,2 | 0,2 | 0,2 |

c' - control (benchmarks)

## MATERIALS AND METHODS

Based on the Department of Hygiene and Ecology #4 of Bogomolets National Medical University and the Institution of Hygiene and Ecology conducted a study of the effects of pesticides, namely (Agil KE (propaquizafop ); Akris CE (dimethenamid-P); Basta 200 (glufosinate ammonium); Fusilade forte 150 к.е. EC (fluazifop-P-butyl); Square PK (diquat dibromide); Euro–Lightning PK (imazamox, imazetapir); Vidblok Plus ME (imazetapir, propakhizafop); Primextra TZ Gold 500 SC к.c. (S-Metolachlor, terbuthylazine); Pledge 50 s.p. (Flumioxazine); Racer KE (flurochloridone); Challenge 600 SC KC (aclonifen); Prometrex KC (Prometryn); Acetogan KE (Acetochlor); Proponit 720 к.e. (propisochlor); Pulsar BP (Imazamox)) on the fatty acid composition (lipid) of sunflower seed by gas-liquid chromatography was studied. Sample preparation and gas chromatographic analysis were performed by the method [8].

Samples for gas chromatographic analysis were prepared as follows: samples of 1-3 grams of seeds were placed in a homogenizer and the resulting homogenate of the seeds was transferred into a 10 ml centrifuged tube and filled with the extraction mixture. Lipid extraction was performed with 5 ml of chloroform-methanol mixture (2: 1 ratio) and kept in the refrigerator for 30 minutes. For better phase distribution, 1 ml of distilled water was added. Next, the lower chloroform phase was selected with a Pasteur pipette and concentrated the resulting liquid by evaporation to a volume of one drop.

Hydrolysis and methylation of higher fatty acids of sunflower seed lipid fatty acids were performed by adding 1% of $H_2O_4$ lipid to dry matter in methanol in an amount of 5 ml and transferring this solution into a 10 ml glass ampoule.

After sealing, the ampoule was carried out hydrolysis and methylation in a thermal bath at a temperature of 85ºC for 20 minutes.

Extraction of ethylated fatty acids was performed twice with hexane-ether mixture (1: 1 ratio) in an amount of 5 ml. To distribute the phases, 1 ml of distilled water was added, then the upper phase was selected with a Pasteur pipette and the combined extracts evaporated to dryness in a stream of nitrogen at t- 45ºC in a water bath. The dry precipitate was dissolved in 40-50 µl of pure hexane and introduced into the evaporator of the chromatograph in the amount of 5 µl.

In the spectrum of sunflower lipids, the 9 most informative fatty acids were identified: C14:0 – myristic, C15:0 – pentodecanoic, C16: 0 – palmitic, C17: 0 – margaric, C18:0 – stearic, accounting for the amount of saturated fatty acids (NLCs), and C18:1 is oleic, C18:2 is linoleic, C18:3 is linolenic, C20:4 is arachidonic, which make up the sum of unsaturated fatty acids (NLCs). Content C18:2, C18:3 and C20:4 are included in the amount of polyunsaturated fatty acids (PUFA) and are defined as irreplaceable.

**Table II.** The fatty acid spectrum of saturated fatty acids of sunflower seeds grown when applying various schemes of chemical protection of plants (in %)

| № of sample | Fatty acids | | | | |
|---|---|---|---|---|---|
| | myristic | pentodecane | palmitic | margarine | stearic |
| 1. | 0,3 | 0,3 | 6,0 | 0,3 | 6,6 |
| 2. | 0,2 | 0,2 | 4,0 | 0,1 | 3,2 |
| 3. | 0,2 | 0,2 | 5,0 | 0,2 | 3,0 |
| 4. | 0,2 | 0,2 | 5,0 | 0,2 | 3,1 |
| 5. | 0,2 | 0,2 | 5,0 | 0,2 | 3,7 |
| 6. | 0,1 | 0,1 | 5,0 | 0,1 | 4,0 |
| 7. | 0,2 | 0,2 | 5,0 | 0,2 | 3,6 |
| 8. | 0,2 | 0,3 | 4,4 | 0,2 | 3,2 |
| 9. | 0,2 | 0,2 | 5,0 | 0,2 | 2,6 |
| 10. | 0,2 | 0,2 | 4,5 | 0,2 | 2,4 |
| 11. | 0,1 | 0,2 | 4,4 | 0,1 | 2,3 |
| 12. | 0,1 | 0,1 | 4,3 | 0,1 | 2,8 |
| 13. | 0,2 | 0,2 | 4,0 | 0,2 | 2,0 |
| 14. | 0,2 | 0,2 | 4,4 | 0,2 | 2,6 |
| 15. | 0,2 | 0,2 | 4,5 | 0,2 | 3,0 |
| 16. | 0,2 | 0,3 | 4,2 | 0,2 | 2,5 |
| 17. | 0,1 | 0,1 | 4,6 | 0,1 | 1,9 |
| 18. c' | 0,2 | 0,2 | 4,7 | 0,2 | 3,2 |
| 19.c' | 0,2 | 0,2 | 4,1 | 0,1 | 2,3 |
| 20.c' | 0,2 | 0,2 | 4,3 | 0,2 | 3,1 |

c' - control (benchmarks)

## RESULTS

The influence of plant protection chemicals on the fatty acid composition of sunflower seed lipids was evaluated (Table I).

Studies have found that the highest percentage of unsaturated fatty acids in sunflower seeds is oleic and linoleic acids. The result of the experiment revealed that the amount of oleic acid (C18:1) in the samples under numbers 9, 11, 14 does not differ from the control parameters; in samples 1, 2, 6, 7, 8, 13, 15, 16 there is a deviation toward the increase from the control data (6.8%, 3.4%, 0.1%, 0.9%, 2.7 %, 0.9%, 0.7%, 1.6% respectively), but they are not statistically significant; in samples 3, 4, 5, 10, 12, 17 there was a tendency to decrease values (1.1%, 0.2%, 3.1%, 1.0%, 0.2%, 1.4% respectively).

The content of linoleic acid (C18:2) in samples 3, 4, 5, 9, 11, 12, 13, 14 did not differ from the control data; in the samples under numbers 1, 2, 6, 7, 8, 15, 16 changes in the concentration of this acid is not statistically significantly lower than the control parameters (12.2%, 2.6%, 0.4%, 1.3%, 2.6%, 0.3%, 0.6% respectively). Studies in numbers 10 and 17 tend to increase the content of linoleic acid (1.0% and 1.7% respectively).

Analysis of the content of linolenic (C18:3) and arachidonic acids (C20:4) in sunflower seeds was not statistically significantly different from the control samples. The largest fluctuations were observed only in sample number 1 for oleic acid by 6.8% and for linoleic acid by 12.2%.

Analyzing the fatty acid spectrum of saturated fatty acids (Table II), namely myristic, pentodecanoic, palmitic, margarine, stearic, after application of plant protection chemicals, established a tendency to increase palmitic content (in samples № 1, 2-7), in samples № 1, 3, 16), margarine (in sample № 1) in relation to the control indicators. However, there was a tendency to decrease the content of myristic acid (in samples No. 6, 11, 12, 17), pentodecanoic (in samples № 6, 12, 17), stearic acids (in sample № 13). However, all these differences were not significant (p> 0.05).

Data provided in Table III, it was found that the total spectrum of fatty acids (saturated, unsaturated and polyunsaturated) in most samples did not differ from the control (p> 0.05).

Only in sample №1 was recorded an increase of 5% in the content of saturated fatty acids and a decrease of 5% and 12% of the amount of unsaturated and polyunsaturated fatty acids, accordingly.

## DISCUSSION

Sunflower is the main oil crop in Ukraine and one of the most important oilseeds in the world. An important aspect of oilseeds is the ability to produce natural and quality oil. But sunflower is mostly grown using crop protection chemicals. Therefore, the requirements for the quality of oilseeds with the use of such means of protection have become a necessary factor today. [5, 9].

**Table III.** The sum of the fatty acid spectrum of solar seeds produced by applying different chemical schemes (in %)

| № of sample | The sum of saturated fatty acids | The sum of unsaturated fatty acids | The sum of polyunsaturated fatty acids |
|---|---|---|---|
| 1. | 13,5 | 86,5 | 68,4 |
| 2. | 7,7 | 92,3 | 77,8 |
| 3. | 8,6 | 91,8 | 82,0 |
| 4. | 8,7 | 91,3 | 80,9 |
| 5. | 9,4 | 91,0 | 81,7 |
| 6. | 9,3 | 91,0 | 79,8 |
| 7. | 9,3 | 91,1 | 79,0 |
| 8. | 8,3 | 91,7 | 77,8 |
| 9. | 8,1 | 91,9 | 80,8 |
| 10. | 7,5 | 92,6 | 83,0 |
| 11. | 7,1 | 92,9 | 82,2 |
| 12. | 7,5 | 92,5 | 82,2 |
| 13. | 6,4 | 93,7 | 81,7 |
| 14. | 7,6 | 92,4 | 81,4 |
| 15. | 8,1 | 91,9 | 80,1 |
| 16. | 7,4 | 92,6 | 79,9 |
| 17. | 6,8 | 93,2 | 83,6 |
| 18.c* | 8,5 | 91,5 | 80,4 |
| 19.c* | 6,9 | 93,4 | 82,4 |
| 20.c* | 8,0 | 93,2 | 82,6 |

c' - control (benchmarks)

In accordance with these requirements, we have assessed the impact to crop protection chemicals, namely that Agil KE (propaquizafop ); Akris CE (dimethenamid-P); Basta 200 (glufosinate ammonium) ; Fusilade forte 150 к.е. EC (fluazifop-P-butyl); Square PK (diquat dibromide); Euro–Lightning PK (imazamox, imazetapir); Vidblok Plus ME (imazetapir, propakhizafop); Primextra TZ Gold 500 SC к.с. (S-Metolachlor, terbuthylazine); Pledge 50 s.p. (Flumioxazine); Racer KE (flurochloridone); Challenge 600 SC KC (aclonifen); Prometrex KC (Prometryn); Acetogan KE (Acetochlor); Proponit 720 к.е. (propisochlor); Pulsar BP (Imazamox) on the spectrum of fatty acid composition of lipids in sunflower seeds.

A number of authors in determining the amount of macronutrients in sunflower oil did not note significant changes in lipid concentration after treatment of the crop with plant chemicals [9-11]. However, there aren't studies that have been performed on the presence or absence of changes in the fatty acid spectrum of the oil after chemical treatment. This was the purpose of our study.

We investigate the probability of changes in the spectrum of fatty acids (FA) 9 of the most informative acids:: C14:0 – myristic, C15:0 – pentodecanoic, C16: 0 – palmitic, C17: 0 – margaric, C18:0 – stearic and separately their sum (sum of saturated FA) as well C18:1 – oleic, C18:2 – linoleic, C18:3 – linolenic, C20:4 – arachidonic (and their amount is also sum of unsaturated FA). In all studied samples the content of saturated FA s: myristic, pentodecanoic,, margarine almost did

not differ from the control data. Increased content of palmitic and stearic fatty acids is observed only in sample №1 (on 36% and 50% respectively), which is an exception and does not carry statistically significant data, in all other samples – the data are not significantly different from the control.

The results of the content of linolenic and arachidonic acids, the amount of unsaturated fatty acids studied did not differ from the control data. Slight changes in both increase and decrease in values were observed in oleic and linoleic fatty acids compared to control data, but they were not statistically significant (not reliable).

## CONCLUSIONS

Thus our research results give reason to believe that the studied pesticides (Agil KE (propaquizafop ); Akris CE (dimethenamid-P); Basta 200 (glufosinate ammonium); Fusi lade forte 150 к.е. EC (fluazifop-P-butyl); Square PK (diquat dibromide); Euro–Lightning PK (imazamox, imazetapir); Vidblok Plus ME (imazetapir, propakhizafop); Primextra TZ Gold 500 SC к.с. (S-Metolachlor, terbuthylazine); Pledge 50 s.p. (Flumioxazine); Racer KE (flurochloridone); Challenge 600 SC KC (aclonifen); Prometrex KC (Prometryn); Acetogan KE (Acetochlor); Proponit 720 к.е. (propisochlor); Pulsar BP (Imazamox)) in various schemes of chemical protection of sunflower crops does not affect the nutritional and biological value of the seeds (the fatty acid composition of sunflower seed).

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ORIGINAL ARTICLE

# NURSES' KNOWLEDGE ABOUT CORONARY ARTERY DISEASE IN AL-NASIRIYAH CITY

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**ABSTRACT**

**The aim**: The present study aims is to assess nurses knowledge between men and women concerning for coronary artery disease patient in AL-Nasiriyah City hospitals, and also to measure the percentage of these knowledge.

**Materials and methods**: A descriptive study design to achieve the objectives of the study. The study has been conducted upon non-probability (purposive) sample of (175) nurses. All nurses who work at medical department were selected based on the study criteria. The questionnaire has been adopted to meet and achieve the objectives of the study. The questionnaire consists of two parts: part one demographic characteristic for students; part two consists of Nurses' knowledge toward coronary artery disease scales. The Validity of the questionnaire has been determined through a panel of experts; Reliability of the Questionnaire was developed by pilot study. The data were analyzed by using (SPSS ver. 24) through the application of descriptive statistics.

**Results:** The results of the study show that the overall nurse's knowledge concerning coronary artery disease majority of the sample have low knowledge with percentage 70%.

**Conclusions:** The study concluding that most gender of the sample is male and most members of the sample have low knowledge of coronary artery disease CAD. The study recommends creating an educational program for newly appointed nurses to increase their knowledge, emphasis on the center for continuing education by conducting continuous courses on coronary artery disease, migrating certificates to a higher certificate through the distinguished channel and increasing their academic achievement

**KEY WORDS:** Nurses, knowledge, coronary artery disease (CAD)

## INTRODUCTION

Coronary artery diseases (CAD), also known as coronary heart disease(CHD) or heart disease (HD) (Wilson and Douglas, 2015) and ischemic heart disease (IHD) [1]. CAD is the most common type of cardiovascular disease (CVD), contributed approximately half of all CVD deaths worldwide. CAD is an important public health problem, among adults with a high morbidity and mortality, and it causes a substantial economic burden to societies [2].It is the leading cause of death for both men and women. More than half of the deaths due to heart disease in 2015 were in men. Each year about 630,000 Americans die from heart disease that's one in every four deaths, it is killed about 366,000 people in 2015.In the United States, every 40 seconds someone has a heart attack. Each minute, more than one person in the United States dies from a heart disease-related event [3]. CAD is the most common type of cardiovascular diseases & it is the major causes of death approximately 4 million deaths resulting from MI occur around the world annually. Every year about 1.25 million infarctions occur in the United States, 50,000 of which culminate in death [3]. Coronary Artery Disease could lead to heart attacks [4]. The hospitalization rate of the patients with CAD has also increased in the recent years, indicating the increasing incidence and recurrence rates of this disease. Despite hopes in reduction of mortality, the sharp increase in patients' admission is becoming a growing concern [5]. In Iraq, the number of CAD is increased as a health prob-lem according to hospitals morbidity data provided by Iraqi ministry of health in 2004 shows a 65% Increase of the hospital admission due to coronary heart disease and smoke. More than

**Table I.** Socio-demographic variables descriptive statistics of the nurses

| Items | Rating | F. | % |
|---|---|---|---|
| Age | 20-29 | 80 | 45.7 |
| | 30-39 | 57 | 32.6 |
| | 40 and above | 38 | 21.7 |
| | Total | 175 | 100.0 |
| Gender | Male | 98 | 56.0 |
| | Female | 77 | 44.0 |
| | Total | 175 | 100.0 |
| Level of qualification | Preparatory | 52 | 29.7 |
| | Institute | 74 | 42.3 |
| | College | 49 | 28.0 |
| | Total | 175 | 100.0 |
| Years of experiences in nursing | Less than 2 years | 61 | 34.9 |
| | 2-10 | 89 | 50.9 |
| | 11 and above | 25 | 14.3 |
| | Total | 175 | 100.0 |

**Table II.** Assessment of nurses' knowledge levels toward CAD

| Items | Rating | F. | % | M.S | Ass. |
|---|---|---|---|---|---|
| Coronary arteries arise from… | Incorrect | 78 | 44.6 | .55 | M |
| | Correct | 97 | 55.4 | | |
| | Total | 175 | 100.0 | | |
| Coronary artery are branches into… | Incorrect | 66 | 37.7 | .62 | M |
| | Correct | 109 | 62.3 | | |
| | Total | 175 | 100.0 | | |
| Result of accumulation of plagues on the myocardial arteries are called | Incorrect | 66 | 37.7 | .62 | M |
| | Correct | 109 | 62.3 | | |
| | Total | 175 | 100.0 | | |
| Ischemic coronary arteries mean… | Incorrect | 54 | 30.9 | .69 | H |
| | Correct | 121 | 69.1 | | |
| | Total | 175 | 100.0 | | |
| Clinical manifestation of coronary artery disease is in from of… | Incorrect | 66 | 37.7 | .62 | M |
| | Correct | 109 | 62.3 | | |
| | Total | 175 | 100.0 | | |
| Acute chronic coronary arteries syndrome… | Incorrect | 104 | 59.4 | .41 | M |
| | Correct | 71 | 40.6 | | |
| | Total | 175 | 100.0 | | |
| is a state that affecting heart muscle and causing it is failure leading to impaired cerebral blood supply causing death… | Incorrect | 103 | 58.9 | .41 | M |
| | Correct | 72 | 41.1 | | |
| | Total | 175 | 100.0 | | |
| Rarely fatty deposits forming less than from the artery cavity cause coronary artery blockage symptom… | Incorrect | 126 | 72.0 | .28 | L |
| | Correct | 49 | 28.0 | | |
| | Total | 175 | 100.0 | | |
| Risk factors that can be controlled are… | Incorrect | 109 | 62.3 | .38 | M |
| | Correct | 66 | 37.7 | | |
| | Total | 175 | 100.0 | | |
| Risk factors that can be uncontrolled are… | Incorrect | 133 | 76.0 | .24 | L |
| | Correct | 42 | 24.0 | | |
| | Total | 175 | 100.0 | | |
| symptoms of coronary artery disease are started when percentage of obstruction is… | Incorrect | 120 | 68.6 | .31 | L |
| | Correct | 55 | 31.4 | | |
| | Total | 175 | 100.0 | | |
| Diagnosis of coronary artery diseases by risk assessment through… | Incorrect | 72 | 41.1 | .59 | M |
| | Correct | 103 | 58.9 | | |
| | Total | 175 | 100.0 | | |
| A test for heart function… | Incorrect | 72 | 41.1 | .59 | M |
| | Correct | 103 | 58.9 | | |
| | Total | 175 | 100.0 | | |
| priorities of nursing intervention of coronary artery patients during pain are… | Incorrect | 60 | 34.3 | .66 | M |
| | Correct | 115 | 65.7 | | |
| | Total | 175 | 100.0 | | |
| priorities of nursing intervention of coronary artery patients through… | Incorrect | 61 | 34.9 | .65 | M |
| | Correct | 114 | 65.1 | | |
| | Total | 175 | 100.0 | | |

| | | | | | |
|---|---|---|---|---|---|
| Encourage patient to… | Incorrect | 133 | 76.0 | .24 | L |
| | Correct | 42 | 24.0 | | |
| | Total | 175 | 100.0 | | |
| Tell the patient routes of treatment are necessary through… | Incorrect | 30 | 17.1 | .83 | H |
| | Correct | 145 | 82.9 | | |
| | Total | 175 | 100.0 | | |
| Goal of nursing intervention of coronary artery patients is… | Incorrect | 157 | 89.7 | .10 | L |
| | Correct | 18 | 10.3 | | |
| | Total | 175 | 100.0 | | |
| The patient can use simple activities gradually after… | Incorrect | 126 | 72.0 | .28 | L |
| | Correct | 49 | 28.0 | | |
| | Total | 175 | 100.0 | | |
| Encourage the patient to… | Incorrect | 42 | 24.0 | .76 | H |
| | Correct | 133 | 76.0 | | |
| | Total | 175 | 100.0 | | |

**Table III.** Overall nurses' knowledge toward CAD

| | Rating | Frequency | Percent |
|---|---|---|---|
| Overall Knowledge | Low | 49 | 27.8 |
| | Moderate | 121 | 68.8 |
| | High | 6 | 3.4 |
| | Total | 175 | 100.0 |

a fivefold increase in outpatient visits with the same diagnosis between 1989 and 1999 [6], and the number of patients who are admitted to Iraqi hospitals in 1989 was 9487 and this number increased in 2010 to 19963 patients [7].

## THE AIM

The present study aims is to assess nurses knowledge between men and women concerning for coronary artery disease patient in AL-Nasiriyah City hospitals, and also to measure the percentage of these knowledge.

## MATERIALS AND METHODS

Descriptive, cross sectional research design is used in this study to assess nurse's knowledge about coronary artery disease. A total of 175male and female nurses were selected using purposive sampling technique to reduce the sampling error and enhance the representation of target population, all these nurses who work at medical department were selected based on the study criteria, and after obtaining consent from them. Study was conducted at Al-Nasiriya city hospitals. A questionnaire has been adopted from [8] to meet and achieve the objectives of the study. The questionnaire consists of two parts; part one demographic characteristics and part two consist of Nurses' knowledge toward coronary artery disease scales. As validity is concerned with the extent to which an instrument corresponds. The content validity of instrument was established through a panel of (5) experts from different specialties. The results of the review of the questionnaire by the experts revealed that all of

the experts agree that 20 items of the study instrument are clear and adequate for the measurement of the phenomenon. The internal consistency of the instrument was determined through the computation of Alpha Correlation Coefficient (Cronbach's Alpha) and the result was high 0.8 it reflects high level of internal consistency and the instrument is reliable and can be applied.

## RESULTS AND DISCUSSION

According to demographic characteristics of the nurses, table (I) shows that about half of the nurses in the study have ages ranging between 20 and 29, this finding agrees with that of [9] who stated that the participants who were age between 25- 30 years-old constitute the majority. And almost all of the nurses are men gender; this finding is supported by Al-Ftlawy (2010) who stated that the males were more than females [10]. In addition, the highest percent of them have graduated from institute; also Jissir (2015) [11] has reported in his research that associate degree of nurses constitute the majority of participated nurses and more than other groups. Lastly, regarding the years of experience in nursing, the study finding indicated that the highest percentage of nurses in the study group between 2-10 years of experiences. This finding was congruent with that of Al-Ftlawy (2010) who concluded that the years of experience in nursing were 1-9 years which represents the highest percentage of nurses [10]. On the other hand, and concerning levels of knowledge of nurses and as knowledge about coronary artery disease is crucial to nurses' previous studies have showed that nurse's knowledge about coronary artery disease is not high it may be low or moderate as what has been found by this study (table 2 and 3). In fact, there are many factors that effect on nurse's knowledge it may related to some aspects of demographic data like residency, age, etc. [12-13]. Level of qualification or the academic degree is the primary source for building a body of knowledge, results of this study revealed that only (28%) are graduated from college and have baccalaureate degree while the rest (72%) of the nurses are preparatory or institute, this result may explain the difference in level of knowledge which is reflected moderate and low [14].

In this study this level of knowledge is may related to level of qualification and years of experience, in this study nurses' years of experience were 2-10 to half of them followed by 2 years of experience or less which can result in less knowledge that could gained from cumulative experience [15-16].

## CONCLUSIONS

Study concluded that the majority of respondents are in the age group 20-29 and most of the sample participants are males in addition, most of the sample members have academic attainment institute and Most of them have 2-10 years of experience. According to their knowledge there was a low level of knowledge of nurses about coronary artery disease CAD despite the fact that most of them are graduated from college or institute.

## RECOMMENDATIONS

Recommendations of this study based on the result and conclusions are: first, it is a necessity to Create an educational program for newly appointed nurses to increase their knowledge, second, Emphasis on the Center for Continuing Education by conducting continuous courses on coronary artery disease, third, migrating certificates to a higher certificate through the Distinguished channel and increasing their academic achievement.

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ORIGINAL ARTICLE

# MOLECULAR EPIDEMIOLOGY OF THE TRANSMISSION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN KYIV ACUTE CARE HOSPITALS, UKRAINE

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**ABSTRACT**

**The aim:** To evaluate the potential of transmission of methicillin-resistance Staphylococcus aureus (MRSA) in Ukrainian acute care hospitals.

**Materials and methods:** We performed a multicenter cross-sectional study. Definitions of HAI were used from the CDC/ NHSN. The susceptibility to antibiotics was determined by disk diffusion method according to the EUCAST. The cefoxitin-resistant isolates S.aureus were analyzed for the presence of the mecA gene and femA endogenous control gene using PCR. The virulence factor encoding genes (lukS-PV and lukF-PV) were detected by PCR.

**Results:** Of 2,421 patients with HAIs caused S.aureus included in the study, 28.7% patients had MRSA. Prevalence of nasal carriage rate of MRSA among healthcare workers (HCWs) was 33.3%. MRSA contamination of hands and uniforms/gowns of HCW were 32.2% and 29.7%, respectively. MRSA contamination in the inanimate environment surfaces in near- and extended patients areas were 26.9%. The predominant MRSA contamination in hospital environment surfaces were: room inner door knob (32.8%), bed rails (28.9%), room light switch (28.9%), chair (27.9%), bedside table handle (20.6%), bedside table (20.5%), and tray table (13.7%). The PVL gene was present in 38.7% of MRSA strains, isolated from patients with HAIs and in 55.7% of MRSA, isolated from environment surfaces in patient area. In addition, the PVL genes were detected in over 56.3% of MRSA isolated from HCWs carrier.

**Conclusions:** The majority of MRSA is acquired during hospitalization. Environmental surfaces may serve as potential reservoirs for nosocomial MRSA and facilitate transmissions via contact.

KEY WORDS: Healthcare infections, MRSA, mecA, Panton Valentine Leukocidine, nosocomial transmission, hospital environmental contamination

## INTRODUCTION

The emergence and spread of Healthcare-associated infections (HAIs) caused by methicillin-resistant Staphylococcus aureus (MRSA) has become a serious public health threat worldwide. MRSA is currently the most commonly identified antibiotic-resistant pathogen in hospitals in many parts of the world, including Europe, the Americas, North Africa and the Middle and Far East. HAIs caused by are MRSA pathogens reported to be causing enormous damage to patients and public healthcare, resulting in increased morbidity and mortality and healthcare costs. The incidence of HAIs caused by MRSA remains high. The estimated that almost 150,000 MRSA infections occur every year in countries of the European Union (EU) and the European Economic Area (EEA), resulting in >7,000 attributable deaths [1]. The burden from infections caused MRSA in the European Union/European Economic Area (EU/EEA) has increased in recent years, especially in the higher prevalence southern and eastern countries [2]. According to the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) and the European Antimicrobial Resistance Sur-

veillance Network (EARS-Net) data, in 2020, nine (23%) of 40 countries reporting data on S. aureus had the lowest MRSA percentages (below 5%). MRSA percentages equal to or above 25% were found in 10 (25%) of 40 countries [3]. The incidence of HAI caused by MRSA in Ukraine ranged from 13.2% [4] to 35.7% [5, 6]. Incidence of MRSA nasal carriage among Ukrainian HCWs was 17-34% [7,8].

For the prevention of HAIs, knowledge about transmission routes is essential. There are multiple transmission routes by which patients may acquire MRSA or other infectious agents that are capable of surviving in the environment. Environmental surfaces may serve as potential reservoirs for nosocomial pathogens and facilitate transmissions via contact depending on its tenacity [9].

Hospital disinfection policies have a major role to play in the control of MRSA. Currently, in Ukraine used the healthcare environment, up to five hundred biocides, mainly for the disinfection of hospital surfaces. However, the effectiveness of most of these biocides is not known. The usage of these biocides in the hospital environment may not be justified and

is detrimental in the long term, for example, by promoting the emergence of bacterial resistance to specific antimicrobials.

Literature data show that anyone patients or healthcare personnel can get MRSA. The risk transmission of MRSA increases with skin-to-skin contact, and shared equipment or supplies in hospitals [10]. Asymptomatic carriers of MRSA are important sources of nosocomial transmission. However, the route of transmission of MRSA in hospitals is not completely understood. In Ukraine, there are no studies examining the transmission of MRSA in hospitals.

## THE AIM

To evaluate the potential of transmission of methicillin-resistance Staphylococcus aureus (MRSA) in Ukrainian acute care hospitals.

## MATERIALS AND METHODS

### STUDY DESIGN AND SETTING

We performed a multicenter cross-sectional study was conducted from January 2021 to December 2021 in eight acute care hospitals in Kyiv, Ukraine. We have included hospitals that are similar in terms of medical equipment, personnel, and laboratory facilities, and number of beds. All participating hospitals were required to have at least 1 infection control professional, a clinical microbiology laboratory with the capacity to process cultures.

### DEFINITIONS

Major and specific HAI site definitions were adapted from the Centers for Disease Control and Prevention's (CDC's) / National Healthcare Safety Network (NHSN) Surveillance Definitions for Specific Types of Infections [11]. Institution of antimicrobial treatment by a physician without microbiological confirmation was not considered to be sufficient for diagnosis of an HAI in any other circumstance. Serologic and antigen test results were not included in case definitions because laboratories in participating hospitals did not have the capability to perform these tests. Cases were described as community-acquired (CA) if MRSA was isolated from patients within on either of the first two days of hospital admission were categorized as isolates from community-acquired (CA) infections, while isolates collected on day three in hospital or later were categorized as isolates of MRSA from hospital-acquired (HA) infections [12].

### DATA COLLECTION

Surveillance data of MRSA strains isolated from patients (colonized/infected), healthcare workers and from inanimate surfaces, were collected on a form specifically designed by the investigators. We analyzed data collected during a 12-month period. As part of this analysis, basic patient data were collected, including age (≥1 yr old), sex, and specimen type. Hospitals also provided consecutive, nonduplicate isolates for molecular typing. All patients admitted to acute care hospitals in study period were analyzed. Data from clinical medical records and micro-bial reports were reviewed. The follow-up of each patient was continued until discharge or death. Contact precautions were implemented over standard precautions for patients with HAIs.

### SAMPLING STRATEGY

Samples for were collected from patients with HAIs and other patients at the time of admission to hospitals that participated in this study, and on the day of discharge. Additionally, we took samples from near- and extended patient (colonized or infected) areas, also from healthcare personnel (nares/hands/uniforms/gowns).

### NOSOCOMIAL TRANSMISSION OF MRSA

Nosocomial transmission events in the hospital were defined as the transfer of MRSA from a colonized patient to another patient who was previously negative, hospital stays that over-lapped with the stay of a colonized patient, and instances of epidemiological linkage. The linkage was defined as either being a roommate of the index patient in a multibed room or being treated by the same attending doctors.

### MICROBIAL METHODS

Bacterial identification and antimicrobial susceptibility testing in this study were carried out with a VITEK 2 system (bioMe´rieux, Marcy l'Etoile, France) and confirmed by the detection of mecA through PCR analysis. Some further antimicrobial susceptibility testing was performed with the disc diffusion method according to the EUCAST guidelines. S. aureus ATCC 29213 was used as a quality control strain for the determination of minimum inhibitory concentrations (MICs). Isolates with either a resistant or an intermediate phenotype were considered non-susceptible for comparison purposes.

### POLYMERASE CHAIN REACTION (PCR)

DNA was extracted from a single colony of each isolate with a QIAamp DNA Mini Kit (Qiagen Gmbh, Germany) according to the manufacturer's guidelines. In this study the cefoxitin-resistant isolates were analyzed for the presence of the mecA gene and femA endogenous control gene using PCR, as previously described. Positive and negative controls were added in each run, we used reference strains that are mecA positive and negative. PCR amplification for Panton-Valentine leukocidin (PVL)-encoding genes (lukS-PV and lukF-PV) was performed on representative isolates, as previously described [13]. Known AMR determinants and the Panton-Valentine leukocidin (PVL) lukF-PV and lukS-PV genes were identified from raw sequence reads [14].

### ETHICS

The study was approved by the Ethics Committee of the Shupyk National Healthcare University of Ukraine. Written informed consent was obtained from all healthcare person-nel and of the patients enrolled or the patient's next to kin.

**Table I.** Demographics and clinical characteristics of HAIs caused by MRSA in acute care hospitals in Kyiv, Ukraine

| Characteristic | No. of HAIa caused by S.aureus | No. of isolates MRSAb | | Specimen origin | | | | Prevalence of HAI caused by MRSA |
|---|---|---|---|---|---|---|---|---|
| | | | | CA-MRSAc | | HA-MRSAd | | |
| | | n | % | n | % | n | % | |
| All patients | 928 | 296 | 31.9 | 64 | 21.6 | 232 | 78.4 | 30.4 – 33.4 |
| **Sex** | | | | | | | | |
| Male | 528 | 160 | 30.3 | 37 | 23.1 | 123 | 76.9 | 28.8 – 31.9 |
| Female | 400 | 136 | 34.0 | 27 | 19.9 | 109 | 80.1 | 32.4 – 35.6 |
| **Age (years)** | | | | | | | | |
| 1–4 | 88 | 14 | 15.9 | 4 | 28.6 | 10 | 71.4 | 14.7 – 17.1 |
| 5–14 | 32 | 3 | 9.4 | 1 | 33.3 | 2 | 66.7 | 8.4 – 10.4 |
| 15–24 | 42 | 6 | 14.3 | 1 | 16.7 | 5 | 83.3 | 13.2 – 15.4 |
| 25–34 | 40 | 5 | 12.5 | 2 | 40.0 | 3 | 60.0 | 11.4 – 13.6 |
| 35–44 | 83 | 11 | 13.2 | 5 | 45.5 | 6 | 54.5 | 12.1 – 14.3 |
| 45–54 | 112 | 18 | 16.1 | 5 | 27.8 | 13 | 72.2 | 14.9 – 17.3 |
| 55–64 | 144 | 52 | 36.1 | 19 | 36.5 | 33 | 63.5 | 34.5 – 37.7 |
| 65–80 | 179 | 77 | 43.0 | 13 | 16.9 | 64 | 83.1 | 41.4 – 44.6 |
| 81≥ | 208 | 110 | 52.9 | 14 | 12.7 | 96 | 87.3 | 51.3 – 54.5 |
| **Patient type** | | | | | | | | |
| Internal | 12 | 1 | 8.3 | 1 | 100 | 0 | 0 | 7.4 – 9.2 |
| Pediatric | 28 | 7 | 25.0 | 3 | 42.9 | 4 | 57.1 | 23.6 – 26.4 |
| Orthopedic | 176 | 56 | 31.8 | 7 | 12.5 | 49 | 87.5 | 30.3 – 33.3 |
| Ear, nose and Throat surgery | 192 | 68 | 35.4 | 17 | 25.0 | 51 | 75.0 | 33.8 – 36.9 |
| Neurosurgical | 97 | 26 | 26.8 | 5 | 19.2 | 21 | 80.8 | 25.4 – 28.2 |
| General surgery | 224 | 97 | 43.3 | 16 | 16.5 | 81 | 83.5 | 41.7 – 44.9 |
| Colorectal surgery | 76 | 18 | 23.7 | 7 | 38.9 | 11 | 61.1 | 22.3 – 25.1 |
| Surgical intensive care | 27 | 7 | 25.9 | 2 | 28.6 | 5 | 71.4 | 24.5 – 27.3 |
| Gynecologic | 64 | 11 | 17.2 | 4 | 36.4 | 7 | 63.6 | 16.1 – 18.4 |
| Obstetrics | 32 | 5 | 15.6 | 2 | 40.0 | 3 | 60.0 | 14.4 – 16.8 |
| **HAI type** | | | | | | | | |
| SSIs | 576 | 197 | 34.2 | 29 | 14.7 | 168 | 85.3 | 32.6 – 35.8 |
| PNEU | 32 | 3 | 9.4 | 3 | 100 | 0 | 0 | 8.4 – 10.4 |
| BSI | 304 | 94 | 30.9 | 31 | 33.0 | 63 | 67.0 | 28.3 – 33.5 |
| UTI | 16 | 2 | 12.5 | 1 | 50.0 | 1 | 50.0 | 11.4 – 13.6 |

Note: aHAI, healthcare infection; bMRSA, methicillin-resistance Staphylococcus aureus, cCA-MRSA, community-acquired MRSA; dHA-MRSA, healthcare acquired MRSA

## STATISTICAL ANALYSIS

The analysis of statistical data was performed using Excel (Microsoft Corp., Redmond, WA, USA). HAIs were analysed by type of infection, which were mutually exclusive. Results are expressed as median, mean ± standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. The primary endpoint was the epidemiology of the methicillin-resistant Staphylococcus aureus (MRSA) isolated from patients and from health care personnel, their resistance to antibiotics. Comparisons were undertaken using Student's t-test and Pearson's chi-squared test or Fisher's exact test for categorical variables as appropriate. Statistical significance was defined as $p < 0.05$.

## RESULTS

### PREVALENCE OF HAIS CAUSED BY MRSA

Of 2,421 patients with HAIs caused S.aureus included in the study, 696 patients had MRSA. The most frequently reported HAI types caused MRSA were Surgical Site Infections (66,6%), Bloodstream infections (31.8%), Pneumonia (1%), and Urinary Tract Infections (0.7%). The overall prevalence of HAIs caused by MRSA was 28.7% (95% confidence interval [CI], 27.8-29.6, $p<0.0001$), and the prevalence of the 4 most frequently recorded types of infections was the following: SSI, 34.2 % (95% confidence interval [CI], 32.6-35.8), BSI, 30.9% (95% CI, 28.3-33.5), PNEU, 9.4% (95% CI, 8.4-10.4), and UTI, 12.5% (95% CI, 11.4-13.6).

**Table II.** Frequencies of healthcare worker MRSA carriage from screening exercises in outbreak settings in acute care hospitals in Kyiv, Ukraine

| Characteristic of departments | Staff screened | Screening method | Frequency of MRSAa isolates |
|---|---|---|---|
| Pediatric | Physicians | Nares | 0 |
| | | Hands | 0 |
| | | Uniforms/gowns | 0 |
| | Nurses | Nares | 6.3% |
| | | Hands | 12.5% |
| | | Uniforms/gowns | 0 |
| Orthopedic | Physicians | Nares | 12.9% |
| | | Hands | 16.1% |
| | | Uniforms/gowns | 11.5% |
| | Nurses | Nares | 31.9% |
| | | Hands | 24.7% |
| | | Uniforms/gowns | 25.8% |
| Ear, nose and throat | Physicians | Nares | 14.2% |
| | | Hands | 11.7% |
| | | Uniforms/gowns | 15.1% |
| | Nurses | Nares | 37.3% |
| | | Hands | 25.9% |
| | | Uniforms/gowns | 18.6% |
| Neurosurgical | Physicians | Nares | 0 |
| | | Hands | 0 |
| | | Uniforms/gowns | 0 |
| | Nurses | Nares | 4.8% |
| | | Hands | 5.1% |
| | | Uniforms/gowns | 1.9%/ |
| General surgery | Physicians | Nares | 24.8% |
| | | Hands | 18.9% |
| | | Uniforms/gowns | 9.6% |
| | Nurses | Nares | 37.3% |
| | | Hands | 35.6% |
| | | Uniforms/gowns | 23.1 |
| Surgical intensive care unit | Physicians | Nares | 0 |
| | | Hands | 0 |
| | | Uniforms/gowns | 0 |
| | Nurses | Nares | 18.8% |
| | | Hands | 22.1%/ |
| | | Uniforms/gowns | 16.8% |
| Gynecologic | Physicians | Nares | 0 |
| | | Hands | 0 |
| | | Uniforms/gowns | 0 |
| | Nurses | Nares | 11.2% |
| | | Hands | 12.8 |
| | | Uniforms/gowns | 4.1% |
| Obstetrics | Physicians | Nares | 2.7% |
| | | Hands | 2.1% |
| | | Uniforms/gowns | 0 |
| | Nurses | Nares | 7.1% |
| | | Hands | 9.8% |
| | | Uniforms/gowns | 2.1% |

Note: aMRSA, methicillin-resistance Staphylococcus aureus

**Table III.** Frequencies of MRSA, isolated from near- and extended patient areas in outbreak settings in acute care hospitals in Kyiv, Ukraine (p<0.05).

| Specimen origin | Number of samples | No. of isolates MRSAa | | CIb 95% |
|---|---|---|---|---|
| | | n | % | |
| Bed rails | 211 | 61 | 28.9 | 27.6 – 30.2 |
| Tray table | 153 | 21 | 13.7 | 12.7 – 14.7 |
| Bedside table handle | 102 | 21 | 20.6 | 19.5 – 21.7 |
| Bedside table | 78 | 16 | 20.5 | 19.4 – 21.6 |
| Chair | 43 | 12 | 27.9 | 26.6 – 29.2 |
| Room light switch | 308 | 89 | 28.9 | 27.6 – 30.2 |
| Room inner door knob | 341 | 112 | 32.8 | 31.5 – 34.1 |
| Total | 1236 | 332 | 26.9 | 25.6 – 28.2 |

Note:
aMRSA, methicillin-resistance Staphylococcus aureus
bCI, confidence interval

**Table IV.** Distribution of the genes in MRSA, isolated from patients with HAI and from screening exercises in outbreak settings in acute care hospitals in Kyiv, Ukraine

| Specimen origin | No. isolates of MRSAb | mecA | | femA | | PVLc | |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % |
| Patients (without HAIa ) nares | 13 | 10 | 76.9 | 10 | 76.9 | 4 | 30.8 |
| HCW nares | 16 | 15 | 93.7 | 15 | 93.7 | 9 | 56.3 |
| HCW hand | 28 | 25 | 89.3 | 25 | 89.3 | 18 | 64.3 |
| HCW uniforms/gowns | 11 | 10 | 90.9 | 10 | 90.9 | 8 | 72.7 |
| Environment surfaces in patient area | 332 | 315 | 94.9 | 315 | 94.9 | 185 | 55.7 |
| bed rails | 61 | 57 | 93.4 | 57 | 93.4 | 27 | 44.3 |
| tray table | 21 | 21 | 100 | 21 | 100 | 19 | 90.5 |
| bedside table handle | 21 | 19 | 90.5 | 19 | 90.5 | 15 | 71.4 |
| bedside table | 16 | 15 | 93.7 | 15 | 93.7 | 11 | 68.7 |
| chair | 12 | 11 | 91.7 | 11 | 91.7 | 8 | 66.7 |
| room light switch | 89 | 85 | 95.5 | 85 | 95.5 | 36 | 40.4 |
| room inner door knob | 112 | 107 | 95.5 | 107 | 95.5 | 69 | 61.6 |
| Total | 400 | 375 | 93.7 | 375 | 93.7 | 224 | 56.0 |

Note:
aHAI, healthcare-associated infection
bMRSA, methicillin-resistance Staphylococcus aureus
cPVL, Panton-Valentine leukocidin

Prevalence of HAIs caused by HA-MRSA and CA-MRSA was 79.2% and 20.8%, respectively. Patient characteristics and prevalence of HAIs caused by HA-MRSA and CA-MRSA are shown in Table I.

## POTENTIAL FOR TRANSMISSION OF MRSA IN HOSPITALS

A total 1,493 strains of S.aureus were isolated from patients without HAIs at the time of admission and on the day of discharge, from healthcare workers (HCW), and samples from patient area surfaces in acute care hospitals. Of these isolates, 26.8% (400/1,493) were MRSA. During the hospital stay among patients without HAIs were identified 13 (15.3%, CI 95%, 13.4-17.2%) nasal carriers of MRSA. The number of positive results of screening for MRSA nasal carriage among patients without HAIs was lower (1 cases) at the time of admission than on the day of discharge (12 cases).

The prevalence of nasal carriage rate of MRSA among healthcare workers (HCWs) was 33.3% (CI 95%, 30.7-35.9%). MRSA carriage rate was highest among nurses 38.7%, (CI 95%, 36.0-41.4) whereas carriage among physicians was 23.5% (CI 95%, 21.3-25.7%). A frequency of HCW MRSA carriage from screening exercises in outbreak settings in acute care hospitals is shown in Table II.

We found high MRSA contamination of hands (32.2%) and uniforms/gowns (29.7%) of HCW. The MRSA contamination of hands and uniforms/gowns was higher in nurses than in physicians. We found significant of MRSA contamination in the inanimate environment surfaces in near- and extended patients with HAI areas. Of 1,236 S.aureus isolated from patient areas, 26.9% were MRSA. A frequency of MRSA, isolated from near- and extended patient areas in outbreak settings shown are Table III.

According to the results of antimicrobial susceptibility tests, all of the isolates were susceptible to vancomycin, linezolid, tigecycline, and teicoplanin (data not shown). For all MRSA isolates, it was found that 96.9% were resistant to penicillin, 68.9% of isolates were resistant to erythromycin, 41.3% were resistant to clindamycin, 63.8% were resistant to tetracycline, 44.3% were resistant to gentamicin, 33.7% were resistant to ciprofloxacin, 36.2% were resistant to levofloxacin, 45.8% were resistant to moxifloxacin, and 15.2% of isolates were resistant to trimethoprim-sulfamethoxazole.

## PREVALENCE OF THE VIRULENCE GENES

In this study the cefoxitin-resistant isolates were analyzed for the presence of the mecA gene and femA endogenous control gene, and the virulence factor encoding genes (PVL-genes). The PVL gene was present in 38.7% of MRSA strains, isolated from patients with HAI. A total of 400 MRSA strains isolated during study period, representing patients without HAI, HCW (nares, hands and uniforms/gowns), and inanimate environment surfaces in near- and extended patient areas, were evaluated. All isolates were identified as MRSA using the oxacillin disc resistance. However, using PCR targeting the mecA gene in S. aureus, only 93.2% (375/400) isolates were confirmed as MRSA. MRSA was characterized by multiplex PCR amplification of the Panton-Valentine leukocidin (PVL) gene and the mecA, and femA gene. The femA gene was positive in all MRSA strains. A total of 56% (224/400) [95% CI 54.5%, 57.5%, p <0.001] MRSA strains were PVL gene positive. Distribution of genes in MRSA isolates arranged by different specimen origin categories shown are Table IV.

## DISCUSSION

This is the first study in Ukraine were to evaluate the potential of transmission of MRSA in Ukrainian acute care hospitals. The overall prevalence of HAIs caused by MRSA in Kyiv acute care hospitals was 28.7%. In this study MRSA colonization/infected was evaluated in isolates obtained from patients both at the time of admission to and at the time of discharge from the acute care hospitals. In our study MRSA colonization was confirmed one patient at admission and 12 on the day of discharge. Many patients were treated by the same attending doctors. The rate of nasal carriage of MRSA among HCWs was 33.3%. We found high MRSA contamination of hands and uniforms/gowns of HCW, and significant of MRSA contamination in the inanimate environment surfaces in near- and extended patients areas.

It can be a sign that hand hygiene and quality of cleaning and disinfection of surfaces in Ukrainian acute care hospitals is still in need of improvement. Our study suggesting that most cases of MRSA are acquired during hospitalization. We believe that there is sufficient evidence to state that inanimate surfaces likely play a role in the transmission of MRSA in Ukraine. Supportive evidence includes hospital environmental cultures demonstrating widespread surface contamination in rooms of many patients colonized or infected with MRSA (Table III and IV) and that hands can become colonized with MRSA (Table II) via patient or environmental surfaces. In addition, this study showed that nasal carriage of MRSA in source HCW and patients, which would increase environmental contamination, has been a risk factor for MRSA acquisition. In our previous study reported the prevalence of MRSA nasal carriage among HCWs in Kyiv, Ukraine was 17% [7]. The present study showed that the rate of nasal carriage of MRSA among HCWs in Kyiv acute care hospitals was 33.3%.

The results of our study showed that the Panton-Valentine leukocidin (PVL)-encoding genes (lukS-PV and lukF-PV) is common among Ukrainian hospital of the MRSA isolates. Our results indicated the PVL gene was present in 38.7% of MRSA strains, isolated from patients with HAIs. We found a high prevalence of the virulence factor encoding genes (PVL-gene) in MRSA, isolated from environment surfaces in patient area (55.7%). PVL-gene were most commonly co-present in MRSA strains, isolated from tray table (90.5%), bedside table handle (71.4%), bedside table (68.7%), chair (66.7%), room inner door knob (61.6%), bed rails (44.3%), and room light switch (40.4%). In addition, the PVL genes were detected in over 56.3% of MRSA isolated from HCWs carrier. This carrier state may also be an important risk factor for transmission MRSA from physicians and nurses to patients and vice-versa [7]. In the current study, the prevalence of MRSA isolates containing PVL genes was higher than in several previous studies in other countries [15-19]. There are increasing reports of MRSA harboring the PVL toxin, which increases strains pathogenicity and their ability to cause infections. Outbreaks of PVL-producing strains have recently been reported worldwide [18, 20]. Problems arise in the treatment of overt infections with MRSA because the antibiotic choice is very limited. In our study all of the MRSA isolates were susceptible (100%) only to vancomycin, linezolid, tigecycline, and teicoplanin.

The combination of the production of PVL, which is a potent toxin involved in severe HAI caused MRSA, with resistance to commonly used antibiotics and ability to spread easily in hospitals, and communities, possess a potential threat to public health. For the control and prevention of HAIs caused by MRSA, knowledge about transmission routes is essential. The transfer of MRSA via patient area surfaces plays a vital role for HAI in acute care hospitals. Therefore, information about the MRSA contamination on surfaces can have direct implications on clinical measures, including hand hygiene guidelines and disinfection strategies.

MRSA can colonize the skin and nares of an HCW without causing sickness (carriers), and in this way, it can be passed on to other susceptible individuals unknowingly. Therefore, in hospitals implementing contact precautions for MRSA carriers is essential for preventing HAIs. For implement effective precautions for avoiding MRSA infections, it is important to clarify when, how, and from whom MRSA is transmitted. It will be extremely difficult to disentangle the contributions of the animate and inanimate reservoirs of MRSA in leading to transient hand carriage of MRSA by HCW. Even though surface contamination may play a role in MRSA transmission, changes in routine disinfection only are unlikely to reduce disease transmission because recontamination of the patient environment area likely is rapid. Clearly, proper hand washing with an antimicrobial agent before and after each contact with patients or their immediate environment and additional contact precautions, ncluding wearing gloves when entering the rooms of patients with MRSA is crucial in preventing transmission of MRSA in hospitals. Nosocomial transmission of the MRSA may easily occur if no appropriate infection control measures are applied on a regular daily basis.

## CONCLUSIONS

Healthcare infections caused MRSA presents a significant burden to the Ukraine hospital system. The majority of MRSA is acquired during hospitalization. There is a potential risk of nosocomial transmission of MRSA. Environmental surfaces may serve as potential reservoirs for nosocomial MRSA and facilitate transmissions via contact. This is due to high environmental contamination with MRSA in the hospital rooms of colonized or infected patients and high the rate of nasal carriage of MRSA among HCWs. Cleaning and disinfection processes must be improved so that there is a reduction in environmental contamination of frequent-contact surfaces. Thorough handwashing and use of recommended barrier precautions are indicated to prevent cross-transmission of MRSA.

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ORIGINAL ARTICLE

# THE INFLUENCE OF TAE-BO ON THE DEVELOPMENT OF MOTOR POTENTIAL OF STUDENTS OF MEDICAL AND PEDAGOGICAL SPECIALTIES AND ITS EFFICIENCY IN THE PROCESS OF EXTRACURRICULAR ACTIVITIES

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**ABSTRACT**

**The aim:** The purpose of this article was to study the impact of Tae-Bo on the development of motor potential of students and its effectiveness in the process of extracurricular activities.

**Materials and methods:** The experiment was conducted in the conditions of the educational process. An experimental group was formed, which was engaged in the traditional educational program of physical education and additionally three times a week, attended training in Tae-Bo. The control group was engaged in the traditional educational program on physical education in HEI. The following methods were used in the work to solve the research tasks: method of analysis and generalization of literature; surveys, questionnaires; methods of pedagogical observation and experiment; method of testing indicators of physical fitness; methods of mathematical statistics.

**Results:** When comparing the indicators of external manifestation of physical qualities of students of control (n = 60) and experimental (n = 60) groups at the end of the formative experiment, statistically significant differences (p <0.05 and p <0.01) were recorded in tests to determine coordination, strength, endurance, flexibility.

**Conclusions:** Thus, the introduction of a developed Tae-Bo training system in the extra-curricular process of HEI students contributed to the positive development of their motor abilities. Experimental work allowed to determine the place and role of modern Tae-Bo training in the extra-curricular process of HEI students.

**KEY WORDS:** fitness technology, students, Tae-Bo, health, healthy lifestyle

## INTRODUCTION

In modern terms, one of the most important issues of educational and educational work is the interest in physical culture. The problem of interest has always required increased attention, as the decline in interest in physical education leads to poor health, bad physical fitness and slow physical development of students [1]. In order to realize this task, it is necessary to introduce new approaches to educational, as well as post-audit, activities in higher educational institutions (HEI), which are based on cultural and health-improving principles and aimed at forming in the student youth a culture of health, values, needs, knowledge, skills and abilities to maintain and enhance health.

One of the most important types of student activities is fitness. Physical education of students of higher education institutions and their extracurricular work on the basis of fitness and fitness technology allows significantly increase their motivation for physical activity, increase indicators of physical development and functional readiness. The current program with the use of Tae-Bo in the educational process in HEI should be aimed at increasing the interest of students in physical education and involving them in systematic physical exercises, as well as for the development of motor skills and health. It contributes to the optimization of the educational process, increases the level of physical fitness, forms a set of sexual and personal needs and abilities that lie in the basis of personal competencies as a student, as well as the teacher, in accordance with the requirements of educational and professional activities.

Such a current trend of physical culture and health activities as fitness, can be seen as a means of improving not only the motor, but also general culture, expansion of the valeological worldview of a young person by such scientists as: O. Petryshyn [2], O. Caykina [3], S. Synytsya, L. Shesterova [4] et al. The introduction of Tae-Bo and fitness technologies in the extracurricular work of students is extremely important, since the issue of organization of extracurricular work is one of the most current problems of modern pedagogical science due to the fact that, improving the quality of higher education is impossible without finding new approaches, forms and methods.

## THE AIM

The purpose of this article was to study the impact of Tae-Bo on the development of motor potential of students and its effectiveness in the process of extracurricular activities.

## MATERIALS AND METHODS

The following methods were used in the work to solve the research tasks: method of analysis and generalization of literature; surveys, questionnaires; methods of pedagogical observation and experiment; method of testing indicators of physical fitness; methods of mathematical statistics.

We have analyzed 79 sources of scientific and methodological literature. The conducted analysis of literary sources by V. Zhamardiy [5], Z. Kozina [6], T. Krutsevych [7] et al. allowed us to highlight the problem of deficit of motor activity of students of Ukraine in curricular and extracurricular time and to identify ways to increase it. Surveys, questionnaires of teachers and students were conducted in order to study the facts that affect physical fitness, attitude of students to a healthy lifestyle as well as the definition of the role of fitness in the extracurricular work of students. The method of pedagogical observation is carried out during the whole experiment on the basis of the Municipal Institution "Kharkiv Humanitarian and Pedagogical Academy" of the Kharkiv Regional Council among 1st year students. We have observed the implementation of the content of the desired experimental fact, we also evaluated current educational achievements, made the selection of tools and methods of conducting extracurricular activities, defined the correspondence of volume and intensity of loading to possibilities of students. The pedagogical experiment consisted in developing and determining the influence of Tai-bo training and its introduction into the process of extracurricular work with HEI students regarding the formation of a healthy lifestyle, as well as the development of motor skills. Pedagogical testing consisted of conducting tests with the help of which we carried out monitoring of adolescents' motor preferences and evaluated their sport interests. Methods of mathematical statistics were used to process the results obtained during experimental studies. At the same time, the task was set to identify patterns in the measurements of the studied indicators and objectively assess the reliability of their changes.

The pedagogical experiment was conducted in the conditions of the educational process on the basis of the Communal institution "Kharkiv Humanitarian and Pedagogical Academy" of the Kharkiv Regional Council. The study was conducted during the 2019-2020 academic year.

Experimental group was formed (n = 60), which constisted from students of 1 year, who in addition to the traditional planned physical education classes additionally, under conditions of extracurricular work, three times a week, attended an hour and a half Tai-Bo training session at the Sports Club "Peremoha" ("Victory"). The control group (n = 60) was engaged in the traditional educational program on physical education in HEI and under the supervision of teachers of physical education attended section classes in their HEI.

The study was conducted in four stages. The first stage (September 2019) was dedicated to the theoretical study of the problem, where modern scientific and methodical materials of domestic and foreign authors were studied and systematized. At the second stage (September 2019 – November 2019) for effective development of Tai-bo training and its introduction into the extracurricular process of HEI students, fitness technology for effective increase of adolescent motor activity and the improvement of their motor skills has been developed and experimentally tested. At the end of this stage, a control test was conducted. The third stage of the study (December 2019 – May 2020) was aimed at identifying the advantages and disadvantages of the established Tai-Bo training in the extracurricular work in HEI concerning the formation of a healthy way of life, adjustment of complexes of special-preparatory exercises and determination of the optimal ratio of amount and intensity of physical activity during classes. At the fourth stage (September-October 2020) the results were summarized, comparative characteristics of the control and experimental groups of the study were established, conclusions were made and work was written.

The Ethics Commission of the Communal institution "Kharkiv Humanitarian and Pedagogical Academy" of the Kharkiv Regional Council has no comments on the methods used in this study.

## RESULTS

To review the results of the experimental work regarding introduction of Tai-bo training in the extracurricular process, the formative stage of the pedagogical process was carried out.

The positive effect of the proposed system was evaluated by the changes in the obtained results of the control indicators of physical fitness during the formative stage of the pedagogical experiment. For this purpose, a control (n = 60) and experimental (n = 60) groups of students of the first year were formed.

The students of the control group attended only physical education classes provided by the curriculum of HEI. Students from the experimental group, in addition to the traditional planned physical education classes additionally, in the conditions of extracurricular work, three times a week, attended an hour and a half long Tai-bo training session. The effect of the proposed Tai-bo training was evaluated by the changes in the results of the control tests at the end of the formation experiment, which, according to the scientific literature, makes it possible to objectively determine the informative indicators of the current state of physical fitness of students. A comparative method was used to analyze the indicators of students of the control and experimental groups. The analysis was carried out in each group separately. The results of the control test of students at the beginning of the pedagogical experiment were compared with the indicators at the end of the experiment and percentage of changes was calculated.

Dynamics of functional characteristics of students' health during the formative experiment was conducted by the

**Table I.** Percentage comparison of functional characteristics of the health of students of the control group (n = 60)

| Control parameters | Indicators at the beginning of the experiment | Indicators at the end of the experiment | Significance of differences | Difference, % |
|---|---|---|---|---|
| | X mean±6 | X mean±6 | p | |
| Pneumothaxometry, L / s | 4.45±0.02 | 4.60±0.05 | > 0.05 | 1.07 |
| Physical condition index, c.u. | 0.53±1.03 | 0.59±1.1 | < 0.05 | 10.17 |
| Evaluation of APCS, c.u. | 2.10±0.01 | 2.23±0.17 | > 0.05 | 4.21 |
| Rufier index, c.u. | 10.90±0.15 | 9.56±0.1 | < 0.05 | 12.29 |

**Table II.** Percentage comparison of functional characteristics of the health of students from the experimental group (n = 60)

| Control parameters | Indicators at the beginning of the experiment | Indicators at the end of the experiment | Significance of differences | Difference, % |
|---|---|---|---|---|
| | X mean±6 | X mean±6 | p | |
| Pneumothaxometry, L / s | 4.45±0.16 | 5.81±0.21 | < 0.01 | 30.56 |
| Physical condition index, c.u. | 0.52±1.07 | 0.75±1.04 | < 0.01 | 44.23 |
| Evaluation of APCS, c.u. | 2.10±0.2 | 1.69±0.09 | < 0.01 | 26.03 |
| Rufier index, c.u. | 10.95±0.31 | 4.3±0.18 | < 0.01 | 60.23 |

**Table III.** Percentage comparison of indicators of the quality of motor abilities of students of the control group (n = 60)

| Control parameters | Indicators at the beginning of the experiment | Indicators at the end of the experiment | Significance of differences | Difference, % |
|---|---|---|---|---|
| | X mean±6 | X mean±6 | p | |
| Shuttle run (4x9 m), s | 11.20±0.01 | 10.97±0.01 | > 0.05 | 2.10 |
| Static balance with closed eyes, s | 19.95±0.14 | 23.00±0.16 | < 0.01 | 15.29 |
| Running 60m from the high stage, s | 9.66±0.04 | 9.63±0.04 | > 0.05 | 0.31 |
| Static force of 14 muscle groups, c.u. | 9.00±1.01 | 9.15±0.91 | > 0.05 | 1.67 |
| Throwing the ball, m | 6.23±0.04 | 8.23±0.08 | < 0.05 | 32.10 |
| Cooper's test, m | 1988.70±0.13 | 2088.14±0.02 | > 0.05 | 5.00 |
| Bending of vertebral column, cm | 8.20±1.1 | 10.56±1.02 | < 0.05 | 28.78 |

**Table IV.** Percentage comparison of indicators of the quality of physical qualities of students of the experimental group (n = 60)

| Control parameters | Indicators at the beginning of the experiment | Indicators at the end of the experiment | Significance of differences | Difference, % |
|---|---|---|---|---|
| | X mean±6 | X mean±6 | p | |
| Shuttle run (4x9 m), s | 11.00±0.01 | 7.67±0.02 | < 0.01 | 30.23 |
| Static balance with closed eyes, s | 20.12±1.00 | 37.25±0.09 | < 0.01 | 85.12 |
| Running 60m from the high stage, s | 10.00±0.03 | 9.61±0.08 | < 0.05 | 3.89 |
| Static force of 14 muscle groups, c.u. | 9.00±0.04 | 12.50±0.07 | < 0.05 | 38.89 |
| Throwing the ball, m | 6.00±0.01 | 11.19±0.01 | < 0.05 | 86.50 |
| Cooper's test, m | 1980.0±0.02 | 2578.95±0.01 | < 0.01 | 30.25 |
| Bending of vertebral column, cm | 8.45±1.01 | 15.63±0.09 | < 0.01 | 85.00 |

following control indicators: determination of inspiratory and expiratory power by pneumothaxometric parameters, physical condition index (by Ye. Pyrohova), assessment of the adaptive potential of the circulatory system (APCS) (by R. Baevsky), assessment of physical workability (Rufier index). Their parameters are given in tables I-II. Comparison of the obtained data allowed to determine the following differences. Inhalation and exhalation power in the control group of studied students during the formative experiment did not reveal statistically significant differences (p > 0.05)

and put 4.55 L / s at the beginning of the educational year (middle level) and 4.60 L / s at the end (middle level). The overall dynamics of the indicator was 1.07%. In the experimental group on the named indicator of the physical criterion formation of a culture of health of students by means of Tai-bo showed significant differences: 4.45 L / s and 5.81 L / s, which makes the dynamics of the transition from the middle to the high level by 30.56% (p <0.01).

The results of determining the index of physical condition by the method of E. Pirogova in the students from control group at the beginning of the formative stage of the pedagogical experiment were 0.53 c. u. (middle level). However, at the end of the school year, there was an improvement to 0.59 c. u, indicating a general increase in the average level by 10.17% (p <0.05). Statistically significant indicators (p <0.01) were found in the experimental group during the study period. Thus, at the beginning of the formative experiment, the index of the physical condition was 0.52 c. u. (middle level), and after the evaluation at the end of the school year it was 0.75 c. u (high level) with an overall improvement by 44.23%. Assessment of the adaptive potential of the circulatory system revealed straining the mechanisms of adaptation of students at the beginning of the experiment: in the control group – 2.14 c. u. (low level) and in the experimental – 2.13 c. u. (low level).

At the end of the academic year, in the students from the control and experimental groups we recorded an adaptation with indicators of 2.22 c. u. (middle level) and 1.69 c. u. (high level), which in the first group decreased by 4.21%, and in the second group there was an increase by 26.03%. Statistical calculations indicated the absence of significant discrepancies (p> 0.05) between the recorded marks of students in the control group, and vice versa, sufficient viability in the experimental group (p <0.01).

Statistically significant results (p <0.01 and p <0.05) were found for the Rufier index for both groups of students. Satisfactory physical fitness at the beginning of the academic year in both groups was recorded: 10.90 c. u. (middle level) – in the control group and 10.95 c. u. (middle level) – in the experimental group. At the end of the study in the control group, the indicators were 9.56 c. u, which made an improvement by 12.29% (middle level). In the experimental group of students, the improvement at the end of the year was 60.23% with the result of 4.35 c. u. (high level).

When comparing the functional characteristics of the health of students of the control (n = 60) and experimental groups (n = 60) at the end of the formative stage of the pedagogical experiment, statistically significant differences were recorded (p<0.01 and p<0.05) in indicators of pneumothaxometry, index of physical condition, estimation of adaptive potential of circulatory system and Rufier index.

When comparing the characteristics of the physical aspect of students' health in the control (n = 60) and experimental (n = 60) groups at the end of the experiment, statistically significant differences were recorded in all studied characteristics (p <0.05 and p <0.01). There is a higher level of manifestation of characteristics of the physical aspect of health among students of the experimental group, and between their output functional indicators of physical health and at the end of the experiment, statistically significant differences and a percentage improvement in the studied characteristics were observed (p<0.05 and p<0.01).

A study of the dynamics of the manifestation of sexual abilities of students during the formative stage of the pedagogical experiment was conducted, which was carried out with the help of a number of pedagogical tests: definition of coordination (shuttle run 4x9 m, static balance with closed eyes), speed (running 60 m from the high stage), strenght (total indicator of static force of 14 muscle groups), speed and power qualities (throwing a stuffed ball 2 kg with two hands from the bottom forward), endurance (Cooper's test), flexibility (bending of vertebral column by leaning forward from a position standing on a gymnastic bench). Their indicators are following (tables III – IV).

Dynamics of the manifestation of coordination abilities of students during the formative stage of the pedagogical experiment was evaluated with the help of the 4x9 m shuttle run and static balance with closed eyes. In the control group of students during the study period, statistically significant differences were found only between the indicators of the static balance (p <0.01).

In the experimental group of students, statistically significant (p <0.01) results were observed between all marks of pedagogical tests. The percentage improvement of the results of the students of the control group in the shuttle run 4x9 m was 2.10% with fixed marks at 11.20 s (average level) and 10.97 s (average level) (p>0.05). In the experimental group of students, the similar increase was 30.23% between the initial and final results: 11.00 s (average level); 7.67 s (high level) (p <0.01).

The results of static equilibrium with closed eyes in the students of the control group at the beginning of the formative experiment had a mark of 19.95 s (low level), and at the end it was 23.00 s (high level), which is by 15.29% (p <0.01) better. In the experimental group, similar indicators of students increased from 20.12 c (low level) at the beginning of the training period up to 37.25 c (high level) at the end of the experiment, which resulted in an overall improvement of 85.12% (p <0.01).

According to the results of the test for speed (running 60 m from the high stage) no significant discrepancies were found between the recorded marks of students of both groups (p> 0.05). During the formative stage of the pedagogical experiment, they remained at the middle level. The percentage improvement of the results of the students of the control group in the 60 m run from the high stage was 0.31% with the recorded marks of 9.66 s and 9.63 s, and of the experimental group – 3.89% with the fixed marks of 10.00 s and 9.61 s, which is explained by the absence of running exercises in the selected system.

The level of appearance of power qualities of students in the process of the formative stage of the pedagogical experiment was determined by static force of 14 muscle groups. Changes in the dynamics of the total indicator of the static relative strength of 14 muscle groups, average

indicators which per 1 kg of weight of students, in the control group we noted an increase of 1.67% – from 9.00 c. u. to 9.15 c. u. in the absence of statistically significant differences (p> 0.05), remained at the middle level. In the experimental group of students, the result increased from 9.00 c. u. (middle level) to 12.50 c. u. (high level), which recorded an increase of 38.89% (p <0.05).

Percentage improvement of the results of the students of the control group in throwing the stuffed ball with two hands from the bottom forward accounted for 32.10% with fixed marks of 6.23 m and 8.23 m (p <0.05) within the low level. In the experimental group of students, a similar improvement between the initial and the final results 86.50% – 6.00 m (low level) and 11.19 m (high level) (p <0.05).

Assessment of endurance dynamics based on the results of the Cooper's test determined the students of the control group at the beginning of the formative stage of the pedagogical experiment mark in 1988.70 m (middle level) with an improvement at the end of the academic year by 5.00% – 2088.14 m (middle level), However, statistical calculations indicated the absence of reliable discrepancies (p > 0.05) between the fixed badges of students of this group. In the experimental group of students, a similar pedagogical test revealed a distance of 1980.00 m (average level) at the beginning of the study, and at the end of the formative experiment – 2578.95 m (high level) in the presence of statistically significant differences (p < 0.01) with a total increase of 30.25%. Estimation of bending of a vertebral column when leaning forward from a standing position on a gymnastic bench found in students of the control group at the beginning of the experiment marks 8.20 cm (low level), and at the end of the school year there was a total increase of 28.78% and amounted to 10.56 cm (middle level) (p < 0.05). In the experimental group of students, similar results at the beginning of the formative experiment were 8.45 cm (low level), and after the second survey an improvement of 85.00% with a mark of 15.63 cm (high level) was recorded (p < 0.01). When comparing the indicators of the appearance of physical qualities of students of the control (n = 60) and experimental (n = 60) groups at the end of the formative experiment statistically significant differences (p <0.05 and p <0.01) were recorded in tests on the definition of coordination, strength, endurance, flexibility.

## DISCUSSION

The introduction of Tai-Bo is due to the availability, popularity, variety of means, its wide stratification, great opportunities to meet the needs for physical activity of young people involved both in an educational institution and outside the classroom.

Therefore, the process of physical education and extra-curricular work of students should make up for the lack of motor activity, which is created in modern conditions of life, and also contribute to the development and normal functioning of all organs and systems of the young organism.

Tai-Bo occupation in the educational process and outside the classroom has a significant developmental effect on the body of students and requires physical education specialists to introduce new pedagogical technologies to solve specific problems of acquiring modern physical education knowledge by students, developing the necessary motor skills, skills and abilities, positive motivations occupation.

## CONCLUSIONS

Thus, the introduction of a developed Tai-Bo training system in the extra-curricular process of HEI students contributed to the positive development of their motor abilities. The changes that took place during the study should guide students to uninterrupted self-improvement and self-creation. In the experimental group, students who attended extra-curricular Tai-bo training, systematically acquired knowledge and practical skills to increase motor activity and the development of motor skills. Organizational and methodological resource provided support and introduction of adapted forms and methods of teaching, aimed at the formation of knowledge, skills and abilities for leading a healthy lifestyle and targeted impact of Tai-Bo tools on improving the level of physical fitness of students. Experimental work allowed to determine the place and role of modern Tai-bo training in the extra-curricular process of HEI students.

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ORIGINAL ARTICLE

# MEANS OF PSYCHOPHYSIOLOGICAL INDICATORS IMPROVEMENT OF FUTURE LAW ENFORCEMENT OFFICERS IN THE PROCESS OF THEIR SPECIALITY TRAINING

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**ABSTRACT**
**The aim:** To investigate the influence of various means of professional training (firearms training, physical training) on the dynamics of psychophysiological indicators of future law enforcement officers.

**Materials and methods:** The research involved 96 17-23-year-old cadets. Three groups were formed: control group (CG, n = 54), the cadets of which studied according to the current educational program; experimental group 1 (EG1, n = 22), the cadets of which additionally attended practical shooting sessions; experimental group 2 (EG2, n = 20), the cadets of which additionally attended training sessions in hand-to-hand combat. The following aspects were studied among psychophysiological indicators: distribution and concentration of attention, short-term memory, mental capacity, emotional stability, anxiety and psycho-emotional state.

**Results:** It was found that the EG1 cadets revealed the most pronounced indicators of attention concentration, emotional stability and psycho-emotional state at the end of the research; the EG2 cadets showed the most prominent indicators of attention distribution, emotional stability and a low level of anxiety. The CG cadets also improved their psychophysiological indicators, but the level of most of them is significantly lower than that of the EG1 and the EG2 cadets.

**Conclusions:** The results of the research confirmed the high efficiency of firearms and physical training means to improve the psychophysiological indicators of future law enforcement officers in the process of their training. The high level of these indicators will ensure their effective performance of service tasks in the process of their future professional activities.

 **KEY WORDS:** psychophysiological indicators, professional training, cadets, firearms training, physical training

## INTRODUCTION

Today, the professional training of modern law enforcement officers includes four types: initial professional training (conducted with newly recruited law enforcement officers for 4 months); service training (carried out in the process of professional activities of law enforcement officers during the entire period of their service); training in higher educational institutions (HEI) with specific training environment (cadets' training lasts 4 years); postgraduate education (utilization tour, advanced training) [1]. Training in the HEI with specific training environment, which are under the Ministry of Internal Affairs of Ukraine, is one of the most important types of professional training during which the formation of future law enforcement officers is carried out [2]. The educational process in the HEI provides an opportunity for cadets to acquire the competencies necessary for their intellectual, moral, spiritual, aesthetic and physical development and effective implementation of future professional activities [3].

Scientists [4, 5] note that the professional activities of modern law enforcement officers take place in extreme conditions, accompanied by many negative factors. Collectively, they result in deterioration of activities, lower physical and mental state of law enforcement officers, can lead to injuries and even disability. This places high demands on the improvement of psychophysiological qualities of law enforcement officers, the effective course of their mental cognitive processes, such as: speed and accuracy of perception of a large amount of information; ability to concentrate for a long time, distribute and switch attention quickly; stable mental capacity; speed, accuracy and timeliness of appropriate responses to stimuli of varying complexity, etc [6]. The proper functioning of these mental processes helps to retain the necessary information in memory, more quickly process the data obtained, quickly switch from one activity to another, which generally contributes to more efficient performance of official duties. According to experts [7, 8], the psychophysiological qualities of cadets i. e. future

law enforcement officers are most effectively developed in the process of practical training. Important means of improving the psychophysiological qualities of cadets in the learning process include the means of firearms and physical training, which are conducted in the HEI in the form of academic disciplines throughout the training period [9, 10]. The analysis of the literature sources has shown that the issue of improving various aspects of the professional activities of law enforcement officers in the process of both firearms and physical training has been studied by many scientists. However, the problems of the impact of firearms and physical training of future law enforcement officers on the dynamics of their psychophysiological indicators, as well as comparing their effectiveness, require further research.

## THE AIM

The aim is to investigate the influence of various means of professional training (firearms training, physical training) on the dynamics of psychophysiological indicators of future law enforcement officers.

## MATERIALS AND METHODS

The research was conducted at the National Academy of Internal Affairs (Kyiv, Ukraine) in 2017-2021. The research involved 96 17-23-year-old cadets, studying in "Law Enforcement" specialty. The duration of the study is 4 years.

To achieve the aim of the research, we formed 3 groups of cadets: control group (CG, n=54), the cadets of which were engaged in accordance with the educational program and attended firearms and physical training sessions as specified in the schedule throughout the training period (2 two hours long training sessions each week both in firearms and physical training) without visiting any additional classes; experimental group 1 (EG1, n=22), the cadets of which additionally attended training sessions of practical shooting at the Academy (3 training sessions per week for 2 hours each) in addition to the mandatory classes in firearms and physical training; experimental group 2 (EG2, n=20), the cadets of which additionally visited training sessions in hand-to-hand combat at the Academy (3 training sessions per week for 2 hours each). The groups were formed at the beginning of cadets' training at the Academy by means of questionnaires on the basis of their own volition.

The research of psychophysiological indicators of cadets was carried out according to the following methods: number finding test (distribution of attention, emotional stability), number operation technique (short term and involuntant memory), "Complex associations" method (thinking features), Anfimov correction task (attention concentration, mental capacity), method of Ch. D. Spielberger, Ju. L. Khanin (state anxiety), method of A. Wessman and D. Ricks (self-assessment of emotional state) [11]. The choice of these methods was determined by

their most complete reflection of the development of psychophysiological qualities, psycho-emotional state and indicators during mental work, which significantly affect the effectiveness of future law enforcement activities of cadets. Testing of psychophysiological indicators of cadets was carried out at the beginning and at the end of their training in the HEI by employees of the department of psychological support.

Research methods include analysis and generalization of scientific and methodological works, pedagogic observation, questionnaire survey, and methods of mathematical statistics.

The research was performed according to the requirements of the Regulations on Academic Honesty at the National Academy of Internal Affairs, which were developed on the basis of Ukrainian experience of ethical rulemaking. The consent to participate in the research was obtained from all subjects.

## RESULTS

The distribution of attention is the ability of a person to allocate attention on a large space, perform several activities in parallel or do several different actions. The analysis of the indicators of the distribution of attention in the cadets according to the test of finding numbers showed that the cadets' indicators of all three groups did not differ significantly at the beginning of the research (p>0.05). In the process of professional training, the indicators of the distribution of attention in all groups significantly improved (p<0.001), which indicates the effectiveness of both firearms and physical training sessions as a means of psychophysiological training of future law enforcement officers (Table I). However, the indicators of the distribution of attention in EG2 cadets were better at the end of the research than in the CG and EG1 cadets for 0.58 points (p<0.05) and 0.27 points (p>0.05) respectively. Whereupon, there was no significant difference between the EG2 and the EG1, as well as the EG1 and the CG (p>0.05). This suggests the most pronounced effect of hand-to-hand combat training to improve the cadets' attention distribution.

Memory is a mental process that consists in consolidating, saving, subsequently reproducing and forgetting past experiences, enabling its re-application in human life and activities. Short-term memory is manifested during the performance of certain activities and is necessary for its implementation in each specified period of time. Involuntant memory is used to memorize information without special learning techniques, while performing activities or working with information. The analysis of the indicators of short-term and involuntant memory, which were studied by the method of operations with numbers, showed that the indicators of the three groups did not differ significantly both at the beginning and at the end of the research (p>0.05). The memory indicators of the cadets of all three groups increased significantly (p<0.001) during their training at the Academy, which allows us to speak about the effectiveness of all the studied means of

**Table I.** Dynamics of psychophysiological indicators of EG1, EG2 and CG cadets in the process of their training at HEI (Mean±SD)

| Stages of the experiment | EG1 (n=22) | EG2 (n=20) | CG (n=54) | Significance level | | |
|---|---|---|---|---|---|---|
| | | | | p1-p2 | p2-p3 | p1-p3 |
| Distribution of attention, points | | | | | | |
| Beginning | 5.11±0.26 | 5.07±0.24 | 5.13±0.19 | >0.05 | >0.05 | >0.05 |
| End | 7.85±0.21 | 8.12±0.19 | 7.54±0.15 | >0.05 | <0.05 | >0.05 |
| p | <0.001 | <0.001 | <0.001 | | | |
| Short-term and involuntant memory, points | | | | | | |
| Beginning | 5.33±0.24 | 5.29±0.23 | 5.41±0.17 | >0.05 | >0.05 | >0.05 |
| End | 7.65±0.19 | 7.68±0.17 | 7.63±0.14 | >0.05 | >0.05 | >0.05 |
| p | <0.001 | <0.001 | <0.001 | | | |
| Thinking features, points | | | | | | |
| Beginning | 4.37±0.27 | 4.32±0.26 | 4.35±0.21 | >0.05 | >0.05 | >0.05 |
| End | 6.76±0.24 | 6.69±0.23 | 6.72±0.16 | >0.05 | >0.05 | >0.05 |
| p | <0.001 | <0.001 | <0.001 | | | |
| Attention concentration, % | | | | | | |
| Beginning | 87.95±0.73 | 88.14±0.69 | 88.04±0.38 | >0.05 | >0.05 | >0.05 |
| End | 96.89±0.66 | 94.97±0.68 | 93.38±0.35 | <0.05 | <0.05 | <0.001 |
| p | <0.001 | <0.001 | <0.001 | | | |
| Mental capacity, c. u. | | | | | | |
| Beginning | 1014.31±19.84 | 1022.75±20.17 | 1027.92±17.46 | >0.05 | >0.05 | >0.05 |
| End | 1372.34±17.97 | 1353.41±18.05 | 1388.53±14.68 | >0.05 | >0.05 | >0.05 |
| p | <0.001 | <0.001 | <0.001 | | | |

Legend: Mean - arithmetical average, SD - standard deviation, p - the significance of the difference between the indicators of each group at the beginning and at the end of the study, p1-p2 - the significance of the difference between the indicators of the EG1 and EG2, p2-p3 - the significance of the difference between the indicators of the EG2 and CG, p1-p3 - the significance of the difference between the indicators of the EG1 and CG

developing psychophysiological indicators of future law enforcement officers.

Thinking is the process of transforming facts, information, emotions, etc. into holistic and orderly knowledge; is a fundamental property of a human being. The dynamics of the indicators of cadets' thinking is similar to the dynamics of memory indicators i. e. a significant ($p<0.001$) improvement of the indicators of all three groups of cadets was revealed during the whole process of cadets' training with no significant difference between groups at the beginning and at the end of the research ($p>0.05$).

Attention concentration is understood as the intensity of concentration of consciousness on the object. The analysis of the indicators of cadets' attention concentration showed that during the training period there was a significant improvement in this psychophysiological characteristic in all three groups ($p<0.001$). At the end of the research, the highest level of attention concentration was found in EG1 cadets. This value (96.89%) was significantly better than that of the EG2 cadets (94.97%) for 1.92% ($p<0.05$), as well as better than that of the CG cadets (93.38%) for 3.51% ($p<0.001$). At the same time, the level of attention concentration in the EG2 cadets was also significantly better that of the CG cadets for 1.59% ($p<0.05$). This shows that additional training sessions in practical shooting is

the most effective way to improve the concentration of attention of future police officers.

The research of mental capacity shows that the level of mental capacity in the EG1, the EG2 and the CG cadets does not differ significantly both at the beginning and at the end of the research ($p>0.05$). The CG cadets revealed the best indicator of mental capacity at the end of the research. Within the training process, the level of mental capacity of the cadets of all three groups significantly ($p<0.001$) improved from low to medium and from medium to high. It has been established that additional sports activities, which promote the development of such strong-willed qualities in cadets as purposefulness, determination, persistence, not only do any harm to the educational process, but also contribute to the improvement of educational activities.

The indicators of state anxiety allowed us to assess the level of cadets' anxiety at a particular time: at the entrance to the Academy and at the end of training in the HEI. State anxiety is characterized by tension, excitement, nervousness. The higher the state anxiety is, the worse are the indicators of attention, coordination and the lower is the effectiveness of educational and professional activities of cadets. The analysis of the indicators of state anxiety shows that the level of anxiety of the cadets of all three groups at the beginning of the research was assessed as

**Table II.** Dynamics of psycho-emotional state indicators of EG1, EG2 and CG cadets in the process of their training at HEI (Mean±SD)

| Stages of the experiment | EG1 (n=22) | EG2 (n=20) | CG (n=54) | Significance level | | |
|---|---|---|---|---|---|---|
| | | | | p1-p2 | p2-p3 | p1-p3 |
| State anxiety, c. u. | | | | | | |
| Beginning | 49.34±0.97 | 50.08±0.94 | 49.57±0.81 | >0.05 | >0.05 | >0.05 |
| End | 32.96±0.75 | 33.27±0.76 | 36.04±0.68 | >0.05 | <0.05 | <0.05 |
| p | <0.001 | <0.001 | <0.001 | | | |
| Self-assessment of cadets' emotional state, points | | | | | | |
| Beginning | 4.79±0.24 | 4.81±0.23 | 4.85±0.19 | >0.05 | >0.05 | >0.05 |
| End | 7.88±0.19 | 8.02±0.18 | 6.77±0.15 | >0.05 | <0.001 | <0.001 |
| p | <0.001 | <0.001 | <0.001 | | | |
| Emotional stability, points | | | | | | |
| Beginning | 5.17±0.27 | 5.09±0.24 | 5.12±0.20 | >0.05 | >0.05 | >0.05 |
| End | 8.45±0.20 | 8.56±0.18 | 7.73±0.13 | >0.05 | <0.01 | <0.01 |
| p | <0.001 | <0.001 | <0.001 | | | |

Legend: Mean - arithmetical average, SD - standard deviation, p - the significance of the difference between the indicators of each group at the beginning and at the end of the study, p1-p2 - the significance of the difference between the indicators of the EG1 and EG2, p2-p3 - the significance of the difference between the indicators of the EG2 and CG, p1-p3 - the significance of the difference between the indicators of the EG1 and CG

"high" and did not differ significantly (p>0.05). During the research period, the level of anxiety in the EG1, the EG2 and the CG cadets decreased significantly (p<0.001); the optimal level of state anxiety was revealed in all groups at the end of the research. Nevertheless, the comparative analysis of the indicators showed that the level of anxiety is the lowest in the EG2 (33.27 c. u.) among other groups (Table II). Whereupon, the difference between the EG2 and the CG is significant (2.77 c. u.; p<0.05), the difference between the EG1 and the CG is also significant (3.08 c. u.; p<0.05). There is no difference between the EG2 and the EG1 (p>0.05), which suggests the effective impact of additional training sessions in both practical shooting and hand-to-hand combat to reduce the level of cadets' anxiety.

The analysis of the indicators of self-assessment of cadets' emotional state showed that if they did not differ significantly at the beginning of the research in all groups (p>0.05), then the level of self-assessment of emotional state was significantly better in the EG1 and the EG2 cadets at the end of the research than in the CG for 1.11 points and 1.25 points respectively (p<0.001) (Table II). At the same time, no significant difference was revealed between the indicators of the EG1 and the EG2 cadets (p>0.05). During the experiment, the level of self-assessment of emotional state in the cadets of all three groups improved significantly (p<0.001), but a more pronounced difference was revealed in the EG1 and the EG2 cadets, which indicates a positive effect of additional shooting and hand-to-hand combat sessions on the emotional state of cadets in the process of their training at the Academy.

The analysis of the cadets' emotional stability showed that a low and significantly the same level of emotional stability was revealed at the beginning of the research in all three groups (p>0.05). The indicators of emotional stability in

the EG1 and the EG2 cadets were significantly better than in the CG cadets at the end of the research (p<0.01). This allows us to say about the positive impact of additional sports activities on the formation of emotional stability of cadets, which will contribute to the effective performance of tasks under stress and under the influence of other adverse factors in terms of future professional activities.

## DISCUSSION

Scientific research [12, 13] and practice of law enforcement show that the current conditions of professional activities of law enforcement officers, especially those who protect public safety and order, provide for constant contact with various segments of the population. They always have to confront the most socially difficult contingent of the population and "problematic" people (who are under the influence of alcohol or drugs; emotionally aroused; mentally ill, etc.). Such persons are characterized by the presence of antisocial attitudes, uncontrollability and aggression, covert nature of criminal activity, confrontation and hostility to government officials. In many cases, service communication is forced and sometimes imposed, it takes place in a conflict of interest and disagreement between the parties, lack of information and time; with a high degree of proneness to conflict. Establishing communication during contact with such a contingent of citizens requires the formedness of appropriate skills in law enforcement officers and a high level of development of psychophysiological qualities [14].

In addition, according to many scientists [15, 16], the conditions of professional activities of law enforcement officers are characterized by irregular working hours, constant psychological and physical overload. They have to constantly apply preventive police and coercive measures while accom-

plishing their official duties. There is a possibility of an attack by aggressive offenders, which can result in injury and so on. All of the above requires an appropriate level of development of such psychophysiological indicators in law enforcement officers as the ability to simultaneously perceive several objects (scope of attention), perform several actions (distribution of attention), focus on one object (concentration of attention), the ability to maintain the required intensity of attention for a long time (stability of attention). In addition, the professional activities of law enforcement officers place high demands on the functions of memory and thinking, emotional stability and emotional state.

The results of studies [17, 18] show that the proper level of development of both physical and psychological qualities of law enforcement officers in the process of firearms and physical training sessions contributes to the body's resistance to negative factors of professional activities, reduces the impact of fatigue and is a determining factor not only of the quality of tasks performance during official activities, but also the formation of the authority of the National Police of Ukraine, increasing public confidence in law enforcement. The results of our research have shown that both firearms training and physical training are effective means of forming psychophysiological indicators of future law enforcement officers in the process of their training in the HEI. At the same time, it turned out that the cadets who additionally attended training sessions in practical shooting revealed more pronounced indicators of attention concentration, emotional state and a low level of anxiety. Moreover, the cadets, who additionally attended training sessions in the hand-to-hand combat revealed a high level of development of such indicators as the distribution of attention, emotional state and a low level of anxiety. The cadets who underwent professional training in accordance with the educational program and who did not attend additional training sessions in firearms and physical training also improved psychophysiological indicators, but their level was lower at the end of training in the HEI, compared to the experimental groups cadets. Our research does not completely solve the problem of forming physically and psychologically prepared future law enforcement officers for professional activities. The obtained results only expand the conclusions of the work of many scientists in this field [19, 20].

## CONCLUSIONS

It was found that the EG1 cadets revealed the most pronounced indicators of concentration, emotional stability and psycho-emotional state at the end of the research; the EG2 cadets showed the most prominent indicators of attention distribution, emotional stability and a low level of anxiety. The CG cadets also improved their psychophysiological indicators, but the level of most of them is significantly lower than that of the EG1 and the EG2 cadets. The results of the research confirmed the high efficiency of firearms and physical training means to improve the psychophysiological indicators of future law enforcement officers in the process of their training in the HEI with specific training environment. The high level of these in-

dicators will ensure their effective performance of service tasks in the process of their future professional activities.

The prospects for future research are aimed at studying the means of psychophysiological indicators improvement of law enforcement officers in the process of their service training.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

REVIEW ARTICLE

# KIDNEY DAMAGE IN PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

**Małgorzata Kępska-Dzilińska, Alena Zhymaila, Jolanta Malyszko**
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**ABSTRACT**
The aim of the review is to present the most common renal complications after HSCT such as acute kidney injury, chronic kidney disease glomerulopathies.
HSCT is a critical therapy for many cancer patients with cancer, as well as patients with some other nonmalignant hematologic disorders and certain congenital immune deficiencies.
Kidney complications after HSCT in a form of acute kidney injury is associated with significant morbidity and worse patient outcome. In addition, risk of chronic kidney disease is also increased following HSCT. It is very important to be aware, prevent, early recognize and treat renal damage to improve kidney and patient survival.

**KEY WORDS:** stem cell transplantation, kidney function, chronic kidney disease, acute kidney injury, glomerulopathy

## INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is widely used in therapy of hematological diseases, cancer and amyloidosis. This is a breakthrough management of treatment-resistant diseases. About 50,000 treatments are performed annually around the world [1]. HSCT is divided into autologous HSCT and allogeneic HSCT. In autologous immunosuppressive HSCT treatment is not necessary. In the following years, thanks to modern treatment, the population of long-term survivors has been increasing. However, those who survive a bone marrow transplant are at risk of complications for many reasons such as cytoreductive therapy, conditioning, exposure to transfusion, infection, graft versus host disease (GVHD), immunosuppressants, antimicrobial drugs, thrombotic microangiopathy [2, 3].

One of these late complications is chronic kidney disease (CKD), which can lead to progressive loss of kidney function [4, 5]. According to the work of Abboud at al. CKD occurs in 13-66% of HSCT patients. CKD is caused by, among other things, chemotherapy treatment, occurrence acute and chronic GVHD [6]. It is worth emphasizing that in this area precise pathological changes, the long-term prognosis and optimal choice of HSCT immunosuppressants have not yet been fully determined.

## THE AIM

The aim of the review is to present the most common renal complications after HSCT such as acute kidney injury, chronic kidney disease glomerulopathies.

## REVIEW AND DISCUSSION

### ACUTE KIDNEY INJURY
Acute kidney failure is a common complication after transplantation. It is estimated that 20% to 75% of HSCT recipients have a complication of acute kidney injury (AKI) during HSCT. AKI risk depends on the type of transplant and conditioning regimen [3]. Renal dysfunction following bone marrow transplantation is presented in Figure 1. The epidemiology and prognosis of renal failure are different for the three main transplant procedures, such as myeloablative autograft, myeloablative allograft and non-myeloablative allogeneic transplant. However, what is common is that mortality increases as kidney failure worsens with each procedure. After allogeneic non-myeloablative HSCT is associated with a lower risk of AKI than myeloablative HSCT. This is related to treatment with high-dose chemotherapy and irradiation of the whole body, which is associated with complications such as nausea, vomiting, diarrhea, inflammation of the mucous membranes and hepatic vein occlusive disease [7,8].

The prevalence of AKI in autologous HSCT it is much lower than in allogeneic mainly due to the lack of acute and chronic GvHD, which may contribute to development AKI and chronic renal dysfunction [9,10]. It should be emphasized that the mortality rate is> 80% in patients with end-stage renal disease who are undergoing dialysis [11,12]. As is well known, acute kidney damage may be in the prerenal, renal and post renal form. Prerenal AKI can be caused by fluid loss – vomiting or diarrhea due to chemotherapy. it can also be caused by tumor lysis syndrome
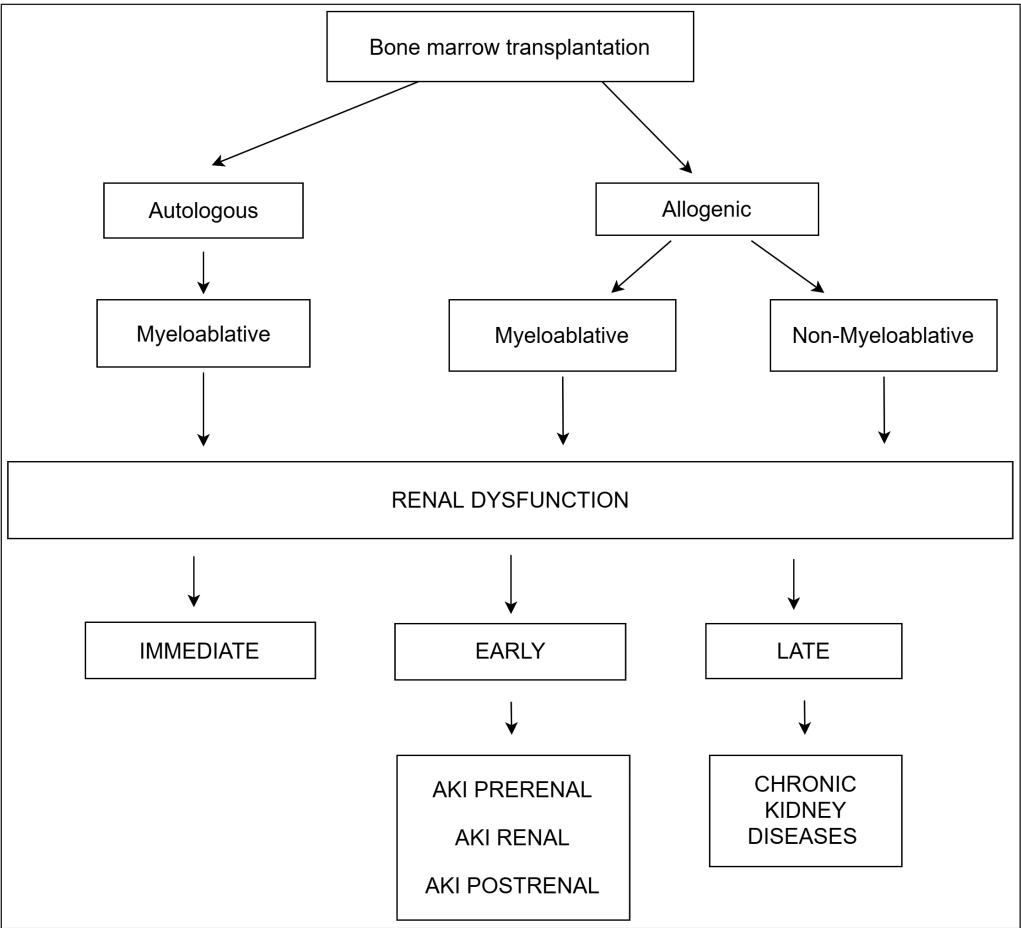
**Fig. 1.** Renal dysfunction following bone marrow transplantation. Different causes for renal dysfunction.

in case of failure to achieve conditional remission. It is also worth emphasizing the role of Capillary Leak Syndrome (CLS) and implantation syndrome (release of pro-inflammatory substances) in the development of AKI. They can show up after autoHSCT and

alloHSCT as a form of fluid retention and fever. Treatment options are steroids

and anti-interleukin-6, which is currently under research [13]. Renal AKI may be caused by sepsis, toxic acute tubular necrosis. The causes of renal AKI can also include the toxic effects of the drugs which cause interstitial nephritis. Adenoviral infections also cause hemorrhagic cystitis which is a common cause of postrenal AKI. 90% of adenovirus patients develop acute kidney injury. the main symptoms are fever, hematuria and pain in the side. Kidney biopsy is necessary to establish a definitive diagnosis [14, 15]. The main treatment is to reduce immunosuppression during viremia to improve T-cell immunity answer. Antiviral therapy with cidofovir is only used for treatment severe cases of haemorrhagic cystitis [16].

## CHRONIC KIDNEY FAILURE

Chronic kidney disease (CKD) is a common complication after HCT. Up to 6-12 months after allogeneic HCT 20% patients develop CKD [17]. A retrospective cohort study by Weiss et al. confirmed that out of 120 patients after HSCT

CKD was found in 65%, and 22% had at least a doubling of the serum creatinine levels within 1 year of the transplant [18]. It is caused by many factors. In various studies, CKD was associated with older age, female gender, use of drugs such as fludarabine, amphotericin B, calcineurin inhibitors. Calcineurin inhibitors have been used in the prevention and treatment of GVHD in most studies were mainly associated with varying degrees of nephrotoxicity [19, 20]. Early research focused mainly on toxicity conditioning [21, 22]. As reported by I. Sekellari at al. there are much more factors associated with kidney damage after HCT. According to this study, the main influencing factors were nephrotoxic drugs, chemoradiotherapy or conditioning, severe infection, and presence of GVHD [23].

Radiation nephropathy is the cause of late renal dysfunction, affecting up to 20% of patients because radiation damages DNA [24]. Radiation can also cause endothelial damage and hemolysis. The incidence of toxicity and kidney damage after total body irradiation is increasing exponentially after a total dose of> 12 Gy [25]. It can be decreased the incidence of late kidney problems by using kidney sheath [26].

Improvements in the prevention of acute GVHD have been achieved in recent years, however, chronic graft versus host disease (GVHD) remains the most common late complication of allogeneic hematopoietic cell transplantation. The prognostic factors of survival in patients

with chronic GVHD have been analyzed in many studies. There were the presence of extensive chronic GVHD, poor performance status, thrombocytopenia, lichenoid changes in skin histology, increased serum bilirubin [27].

## NEPHROTIC SYNDROME AND GLOMERULONEPHTITIS

In patients after HCT, attention should also be paid to the development of nephrotic syndrome. Nephrotic syndrome and proteinuria are rare symptoms of graft versus host disease (GVHD). Only a few reports of cases of glomerulonephritis and nephrotic syndrome can be found in the literature.

Etiology and pathogenesis nephrotic syndrome after HSCT in patients witj chronic GVHD remain unclear [28]. The most common histological diagnoses are membranous nephropathy (MGN) and minimal change disease [29]. MGN is poorly understood. Hiramatsu at al. examined 830 patients (621 patients receiving umbilical cords umbilical cord blood transplant (UCBT) and 208 patients after allogenic bone marrow transplant) undergoing HSCT at Toranomon Hospital from 2000 to 2012. MGN was diagnosed in 5 patients after UCBT (MGN was not found in none after bone marrow transplantation) and has occurred concurrently with chronic graft versus host disease after cessation of immunosuppression. After treatment with immunosuppressants and angiotensin converting enzyme inhibitors was achieved complete remission after approximately 12 months in all patients [29].

Momoki et al. studied data of 1175 patients undergoing allogeneic HSCT (period 1986 to 2013). Nephrotic syndrome developed in 9 (7 men and 2 women). Average time by the time of diagnosis of nephrotic syndrome is 24 months after HSCT. If we look at the histological type (found after kidney biopsy), membranous nephropathy has become 8 (89%) of cases, a type of minimal change in 1 case [30]. Gomez-Garcia et al. reported 2 cases of nephrotic syndrome in the course of chronic GVHD in patients after allogeneic HSCT [31].

## CONCLUSIONS

HSCT is a critical therapy for many cancer patients with cancer, as well as patients with some other nonmalignant hematologic disorders and certain congenital immune deficiencies. Kidney complications after HSCT in a form of acute kidney injury is associated with significant morbidity and worse patient outcome. In addition, risk of chronic kidney disease is also increased following HSCT. It is very important to be aware, prevent, early recognize and treat renal damage to improve kidney and patient survival.

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REVIEW ARTICLE

# HISTORY OF BIOCHEMISTRY IN LVIV

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**ABSTRACT**
**The aim**: The purpose of this literature review is to shed light on the development of biochemical knowledge in the Lviv region and on prominent figures in the development of biochemistry during the Second World War.
**Materials and methods**: Review of literature published before 2020. We searched the literature using the search terms 'biochemists', ' Lviv National Medical University', 'second World War'.
**Conclusions:** The development of biological research in Lviv can be divided into two historical stages: 1) from the beginning of the founding of Lviv University in 1661 to the First World War; 2) between the First and Second World Wars and after the Second World War.

Biochemical research was initiated at the Medical Faculty of Lviv University. In 1939, the Lviv State Medical Institute was established on the basis of the Medical Faculty of the University, where a powerful department of biochemistry functioned, which was headed by a worldclass biochemist – Jakub Parnas.

**KEY WORDS:** biochemists, Lviv National Medical University, second World War

## INTRODUCTION

Biochemistry is a relatively young science that emerged at the turn of the XVIII and XIX centuries, but its roots go back to antiquity. The natural desire of people to understand the causes of diseases and find a cure for them has aroused interest in the processes that occur in living organisms. The use of new drugs and treatments, the most complex surgeries and resuscitation measures require constant biochemical monitoring of the body. Methods of biochemical analysis in the clinic are used to diagnose or study the dynamics of the pathological process. Diagnosis of acute diseases of the liver, kidneys, pancreas, heart, hereditary diseases, beriberi, intoxication, etc. is impossible without biochemical studies.

The first stages of biochemistry coincide with the development of organic chemistry. Later, with the development of synthetic chemistry of carbon compounds, the term "organic chemistry" acquired its modern meaning – the chemistry of carbon compounds, and the science that studies the chemical composition of living organisms and chemical processes occurring in them, began to be called physiological and then biological chemistry. Biological chemistry studies not only organic but also mineral compounds contained in organisms, as well as their role in biological processes.

A significant contribution to the development of biological: science has been made by Ukrainian scientists. In particular, Academician of the USSR Academy of Sciences V.O. Belitzer, who owns scientific developments in the field of biochemistry of oxidative reactions, protein chemistry, is one of the founders of the doctrine of oxidation of phosphorylation; Academician of the National Academy of Sciences of Ukraine M.F. Guliy, who studied the problems of balanced nutrition and carbohydrate metabolism in muscles, studied the relationship between purine metabolism and malignant growth, the regulation of protein and lipid biosynthesis, made a fundamental contribution to modern compounds. in heterotrophic organisms; it is directly involved in the emergence of native molecular biology and immunochemistry.

I.Ya. Gorbachevsky first synthesized uric acid, discovered the enzyme xanthineoxidase. Corresponding Member G.V. Donchenko is known for his achievements in the field of biochemistry of vitamins and coenzymes, in the search for ways of regulation by low-molecular biologically active compounds of intracellular metabolism in humans and animals in normal and various pathologies. in specialized cell structures. Academician of the NAS and AMS of Ukraine SV Komisarenko develops mechanisms of molecular immunology. Corresponding Member of the Academy of Medical Sciences of Ukraine Yu.I. Gubsky owns scientific developments in the field of xenobiochemistry – the direction of research of transformation and molecular mechanisms of physiological effects of foreign substances, molecular pharmacology.

However, there is practically no data in the literature that would cover the development of biochemistry in particular in Lviv and about prominent figures in the history of biochemistry during the Second World War.

## THE AIM

The purpose of this literature review is to shed light on the development of biochemical knowledge in the Lviv region and on prominent figures in the development of biochemistry during the Second World War.

## MATERIALS AND METHODS

Review of literature published before 2020. We searched the literature using the search terms 'biochemists', 'Danylo Halytsky Lviv National Medical University', and 'second World War'. The work envisages measures to enure human health, human dignity and moral and ethical standards in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and relevant laws of Ukraine (opinion of Danylo Halytsky Lviv National Medical University Bioethics Commission).

## REVIEW AND DISCUSSION

During the search, 40 publications were analyzed. Each publication was evaluated individually, and only those that contained original data and photographs were included. A total of 20 publications were processed for viewing.

Sccientific research in various fields of biology in the western region of Ukraine was conducted mainly at the University of Lviv. The history of Lviv University dates back to the seventeenth century. In the sixteenth and seventeenth centuries the centers of cultural life in the Ukrainian lands were church fraternities.

Through the support of the burghers and the clergy, they contributed to the spread of ideas of humanism, the development of science and schooling. The oldest in Ukraine was the Stavropigian Brotherhood in Lviv, which became a prominent Ukrainian cultural center. Since 1586, a fraternal school has been operating in Lviv, which was a secondary school. It has studied Church Slavonic, Creek, latin, Polish, mathematics, grammar, rhetoric, astronomy, philosophy and other disciplines. Members of the Lviv Brotherhood planned to turn this school into a higher education institution.

According to the articles of the Hadiach Agreement (1658) between Ukraine and the Commonwealth, the Polish government promised in the future to open two higher education academies in Ukraine: one in Kiev and the other where it would find a suitable place for it. The academies were promised the same university rights as the rights as the University of Krakow enjoyed. The Jesuits appeared in Lviv at the end of the sixteenth century, and in 1608 opened a high school there. The Jesuits understood the possibility of establishing a fraternal school in Lviv, so they constantly sought to transform their college into an academy. After repeated reguests, King Jan II Casimir signed a diploma on January 20, 1661, which gave the Jesuit college in Lviv the title of university with the right to teach all the then university disciplines, awarding the degrees of bachelor, license, master and doctor (Fig.1). The university consisted of two

departments (faculties) – philosophical and theological. Historical sources testify that in 1667 about 500 students studied at the philosophical and theological faculties, and the teaching process was provided by eight teachers.

The education prices at the University of Lviv was conducted according to the Jesuit school curriculum developed at the end of the 16th century. Changes to this program began to be made only in the middle of the eighteenth century. The Faculty of Philosophy studied Aristotle's philosophical system, which was the essence of lodic, physics, and metaphysics; physics also considered elements of mathematics, astronomy, biology, meteorology, and psychology and ethics in metaphysics. The teaching philosophy department lasted two to three years.

After graduating from this department, it was possible to obtain a obtain a theological education. The teaching department lasted four years. Here were the history of the church, the Old and New Testaments, dogmatic and moral thelogy, canon law, casuistry, ancient Hebrew. All university disciplines were taught by professors.

After the dissolution of the Jesuit Order in 1773, the University of Lviv was closed. However, until the University closed in 1773, a full-fledged medical faculty was never formed.

The first professor of chemistry and botany at Lviv University was Burkhardt Zatsbert Shiverek (1742–1807). B. Shiverek's main services are the study of the mineral waters of the Precarpathian Mountains and the establishment of a botanical garden.

During the second half of the nineteenth century there was an expansion of the university premises. Since 1851, the University has been housed in a building on the street Nicholas (now Grushevsky St.). In 1891 on the project of atchitect Y. Brownsoin on the street. A separate building for chemical, geological, mineralogical and pharmacological institutes was erected in Dluhosha (now St. Cyril and Methodius St.). In 1894, the construction of a building for the newly established Faculty of Medicine (Pekarska Street) was completed.(Fig. 2,3). Almost until the end of the nineteenth century there were three faculties at the Universioty: Law, Philosophy and Theology. The Faculty of Law was a leading university in both the number of students and teachers and the state priorities. In November 1891, the Austrian Emperor Franz Joseph I issued an order to open the Faculty of Medicine, which was solemnly September 9, 1894.

During the years 1891-1898 new educational buildings and clinical bases of the University were built and started to function: anatomo-physiological, pathological, anatomical, chemical, obstetrics and gynecology, surgery, internal diseases, dermatovenereoljgy, otolaryngology and others.

In November 1918, the Polish Ministry of Education, which after the collapse of the Austro-Hungarian Empire, became Galicia, granted the University the name of the Polish King Jan Casimir.

In the 1970s, Ivan Franko, a world-renowned Ukrainian thinker, writer, scientist, translator, psychologist, political and public figure, one of the geniuses of Ukraine who be-

**Fig. 1.** Lviv University in 1661



**Fig. 2.** Emblem of Lviv Medical University



**Fig. 3.** Lviv Medical Institute in 1784

came a "titan of labor," studied at the Faculty of Philosophy at Lviv University. By a decree of January 8, 1940, the Presidium of the Supreme Soviet of the USSR gave the name of Ivan Franko to Lviv State University.

In 1939, after the entry of Western Ukrainian lands into the Ukrainian Soviet Republic, the Faculty of Medicine of Lviv University was separated from the University and reorganized into Lviv State Medical Institute with two faculties: medical and preventive and pharmaceutical. Since 1961, along with national training, the University has been providing training to foreign nationals from Europe, Asia, Africa and America. On October 17, 1996, by decree of the Cabinet of Ministers of Ukraine №1262 the Lviv State Medical Institute was granted the status of a higher educational institution of the IV level of accreditation and was renamed Lviv State Medical University. On October 21, 1998, the University was named Danylo Halytsky Lviv National Medical University, the first King of the Halych-Volyn state.

Among the biochemists who come from the western region of Ukraine, the figure of Ivan Gorbachevsky stands separately. Although his true career growth took place outside Ukraine and was significantly slowed down by the events of the First World War, I. Gorbachevsky never betrayed his Ukrainian roots for a moment [1-5]. Ivan Gorbachevsky is a prominent Ukrainian scientist in the field of organic chemistry and biochemistry (Fig. 4) Also known as a political and educational figure.

Ivan Yakovych was born on May 15, 1854, in the village of Zarubyntsi, which is in Zbarazhchina, in the family of a priest. His path to science was defined in the Ternopil Gymnasium. Here, the future scientist showed great interest, agility and love for the knowledge and his history of his native people.

After graduating from high school, he studied medicine at the University of Vienna. While still a student, he was involved in social and scientific activities. Together with M.Dragomanov, he was chairman of the "Sich" Student Society and began research in medical chemistry.

He graduated in 1877 with a Doctor of Science degree from the University of Vienna. He worked in Vienna at the Chemical Institute and later at the Physical Institutes. In 1882 he synthesized uric acid for the first time in the world, established the source and ways of its formation in the body. In 1883 I.Ya. Gorbachevsky worked at Charles University in Prague.

In 1898 for successes in scientific activity I.Ya. Gorbachevsky was honored with the highest award of Austria-Hungary – the Order of the Iron Crown. In 1911 he was a candidate for the Nobel Prize in Physiology and Medicine. In 2004, UNESCO proclaimed with the 150th anniversary of his birth). In 1900 he participated in the International Medical Congress in Paris, where he headed the Ukrainian delegation. During the congress, he was elected vice president of the chemical section. Hororary President of the Ukrainian Medical Society (1910), initiator and organizer of the First (1926) and Second (1932) Ukrainian Scientific Congresses in Prague.

The credibility and veneration of a scientist in the Czech Republic is evidenced by the fact that he is elected a member of the Sanitary Council of the Czech Kingdom.

I.Gorbachevsky participated in the creation of a monument to the fighter for the will of the Czech Republic Jan Hus, whose discovery was entrusted to the Ukrainian Ivan Gorbachevsky.

In 1917-1918 I.Ya.Gorbaczewski was appointed Austria's first minister of public health. In this post, he conducted a survey of Eastern Galicia and found the situation: poverty of the people, need, hunger, devastation, lack of hospitals, doctors, medicines.

In 1919 he was considered a professor at the Department of Chemistry, which he founded at the Ukrainian Univer-

**Fig. 4.** Gorbachevsky Ivan Yakovych



**Fig. 5.** Stanislav Ludwig Philip Bondzinski



**Fig. 6.** Vaclav Morachevsky (1867-1950) (center). The late 40's



**Fig. 7.** Parnas Jakub Karol



**Fig. 8.** Sobchuk Bogdan Antonovych
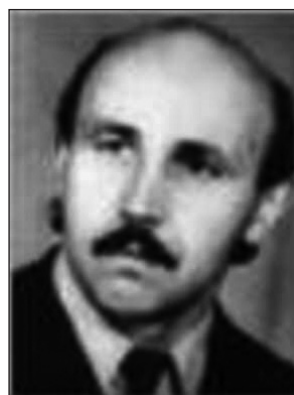


**Fig. 9.** Shlemkevych Mykhailo Petrovych



**Fig. 10.** Tymochko Mykhaylo Fedorovych



**Fig.11.** Sklyarov Alexander Yakovych

sity in Vienna, in 1921 – as a professor, and in 1923– as the rector of the Ukrainian University in Prague.

Together with I.Pulyuy, he organized a "Ukrainian" community in Prague and created a student assistance fund. Since 1925 – academician of the All-Ukrainian Academy of Sciences (Kyiv).

He started his scientific activities at a young age. The first publication was published in 1875, dedicated to the nerve on which the balance of a person depends. He is one of the first to prove that amino acids are an integral part of proteins.

Scientist has also worked productively in the fields of general chemistry, epidemiology, forensic medicine, toxicology.

The participation of I.Gorbachevsky in the scientific life of the Czech Republic and Slovakia is so significant that in some Czech encyclopedias it is noted that he was a Czech scientist of Ukrainian descent.

However, Professor I.Ya. Gorbachevsky never forgot his ancestry. In 1924 he prepared several textbooks on chemistry – "Inorganic chemistry," "Organic chemistry," "Physiological chemistry." The books were written in Czech and Ukrainian. One copy of his "Organic Chemistry" is in the Ternopil Museum of Local History as a gift from a sister of a scientist from the village of Zarubintsi.

I.Ya. Gorbachevsky is the author of 66 scientific works. Most of them have not lost their scientific importance today and their priority is recognized by the scientific community of the world. Many forces I.Ya. Corbachevsky invested in the creation of chemical terminology. He is also the author of more than 100 scientific papers in the field of sanitation.

He was a man of simple temper: gentle, good-natured. With colleagues and acquaintances, he was extremely modest and approachable. He spoke in a quiet voice, unpretentious and simple, with some special attention to the interlocutor.

According to the characteristics of people who personally knew Gorbachevsky, he was tall, good-looking, with a noble face, with clear eyes, kind and sensitive, always ready to help.

He constantly emphasized his Ukrainian origin.

I.Ya. Gorbachevsky was acquainted with Alexander Barvinsky and his son Vasily (composer), Vladimir Hnatyuk (ethnographer), Ivan Pulyu (mathematician).

Out of a sense of great reverence he treated D.I. Mendeleyev, maintained close contacts, made friends, corresponded with eminent scientists, statesmen and church figures, artists, writers, in particular with Lesya Ukrainka. It was on the recommendation of Ivan Yakovich that his cousin I.I. Bilynsky – a pharmacist from Cairo–helped treat the

brilliant poetess in Geluan, as well as the countryman from the Ternopil region – singer of worldwide fame – Solomiya Krushelnytska, the historical Cossacks –D. Yavornitsky and other.

The Medical Faculty of Lviv University, which opened in 1941, invited 88-year-old professor Ivan Gorbachevsky to work, but the death of a prominent scientist and public-political figure prevented it. I.Ya. Gorbachevsky died on May 24, 1942 at the age of 88. He was buried at St. Matthew's Cemetery in Šarka near Prague.

The name of Professor Ivan Gorbachevsky is a symbol of a true scientist, a patriot who has devoted his entire life to science, service to the people, the ideals of humanism. Among many other great people of Ternopil Ivan Gorbachevsky glorified our region. By the resolution of the Cabinet of Ministers of Ukraine № 303 of July1, 1992 the name of Ivan Yakovich Gorbachevsky was given to the Ternopil National Medical University. In 2004, a monument to Ivan Gorbachensky was erected near the Morphological Building of this university.

The Department of Biological Chemistry was founded in 1894 at the Medical Faculty of Lviv University by Professor Vladislav Nemilovich (1863-1904), who in 1891 came to Vienna from the University of Vienna to work as an Assistant Professor of Pharmacognosy and at the same time lectures on chemistry.

From 1906 to 1919 the department was headed by Professor Stanislav Ludwig Philip Bondzinski (1862-1929) (Fig. 5). From 1919 he headed the Department of Phisiological Chemistry at the University of Warsaw, and was also the organizer of the Academy of Medical Sciences (Poland) and its first president.

S. Bondzinski was born on April 30, 1862 in Poland. In 1881-1883 he studied at the Faculty of Mathematics and Natural Sciences of Warsaw University, the Faculty of Philosophy in Lviv (1883), Bern (Switzerland) (1885), from 1892 at the Medical Faculty in Zurich, 1895–Heidelberg. In 1887 he received the title of Doctor of Philosophy ("Ueber Sulfhydrylzimmtsaure und einige ihrer Derivate". Sitzungsberichte d.Akad.d.Wiss.in Wien i Monatsheft fur Chemie; Bern, Switzerland) and in 1895–the title of Doctor of Medicine for the performance of work – "Prace te dotyczyty zachowania sie w ustroju kwasu salicylowego, scladnikow zolci, w szegolnosci cholesteryny, w przewodzie pokarmowym, wreszcie nieznanych dotad produktow roskladu bialka w uskoju" in Heidelberg. In 1897-1898 he foundedand became the first head of the Institute of Food Research (Krakow).

For a short time from 1919 to 1922 the department was headed by Professor V. Morachevsky, the future rector of the Medical Veterinary Academy in Lviv (Fig.6). Vaclav Morachevsky  (1867-1950) was born on November 27 in Warsaw. After graduating from high school in Warsaw from 1885 to 1889, he studied chemistry at the Zuruh Polytechnic, and from 1889 to 1894 studied medicine at a local university. In 1918 V. Morachevsky underwent a medical chemistry habilitation at Lviv University. [6,7].

From 1921 he began to work as a professor at the Medical Veterinary Academy in Lviv.[8-13]. Scientific papers: author of about 180 scientific papers.

The object of scientific research of the department under the direction of prof. S. Bondzinsky was the so-called oxyproteic acid, a protein metabolism product that is excreted in the urine in small quantities. Prof. S. Bondzinsky also studied bile pigments and products of their metabolism, determined the composition of phosphorus in the bones of teeth, cholesterol metabolism in the body, the influence of alcohol on metabolic processes, studied the composition of milk fats, metabolism of caffeine and theobromine in the human body, synthesized the drug "tanabine,"synthesized and conducted a clinical study of acetylsalicylic acid and its esters. Scientific works: author of about 30 scientific papers.

The second stage of biology development in Lviv in the period between World War I and World War II was marked by a shift of emphasis in biological research from the branches of the sciences dominated by obswrvational and descriptive approaches (botany, zoology) to the branches of science with the extensive use of experimental methods, in particular instrumental approaches (biochemistry, normal and pathological physiology, microbiology). Prior to this transition, scientists also encouraged the revolutionary discoveries in the field of physiology and medicine, for which their authors in the early XX century:were awarded the Nobel Prize. In addition to the personal prestige of the Nobel laureate, who gave high prestige to fellow scientists and society, such awards usually opened new directions for scientific progress. It is worth mentioning some Nobel laureates from beginning of XX century and until the end of World War II, whose work contributed to the development of new industries in biology and medicine (in brackets indicate the year of the Nobel Prize): Robert Koch (1905), Ilya Mechnikov (1908), Paul Ehrlich (1908), Archibald Hill (1922), Otto Mayerhof (1922), Karl Landsteiner (1930), Otto Warburg (1931), Thomas Morgan (1933), Albert Stcent-Gyorgyi (1937), Alexander Fleming (1945). Of course, this is by no means a complete list of Nobel laureates who have started new scientific fields in biology and medicine. Experts believe that among the biology scientists who worked in Lviv, the parasitologist Rudolf-Stefan Weigl (creator of the typhoid vaccine) and biochemist Yakub Parnas (who introduced vagomyphalysis in the pathway of Elymobilism), were closest to the Nobel Prize Parnassus).

Another feature of the development of biology during this period was the transition from research conducted by individual scientists, or one scientist with one or two assistants, to the research of large scientific teams, led by a scientific leader, who was the head of the laboratory or department. During this period, many scientific school emerged, an example being the school of the eminent Lviv. [14-20] (Fig. 7).

The world-famous biochemist Jacob Parnas was a native of Lviv region. J. Parnas 28 (according to other data 16) January 1884 in Lviv region near Drohobych, in the village Mokryany Podgayets Country in the landownet's family. There is still debate over the exact birthplace of Jakub Parnas. Polish scientist are of the opinion that Parnas was born

in the village Mokryany of Ternopil region, as it is stated in many scientific and biographical references. Ukrainian biochemist scientist prof. Ivan Golovatsky conducted a special study and found no such village in the Ternopil region (Prof. R. Stoyka) confirmed it. Instead , v. Mokryany is located near Drohobych.

After graduating from Lviv Gymnasium Stanislav Staszyc Jakub Parnas (family name Parnes) studied chemistry at the Berlin-Charlottenburg Polytechnic, the University of Strasbourg and the Zurich Polytechnic (1902-1907), and received his doctorate in Munich (1907). From 1907 to 1916 Parnas worked as an associate professor at the Institute of Physiological Chemistry in Strasbourg, from 1910 to 1911 he was an employee of the Zoological Commission in Naples, in 1914 he became the head of the biochemical laboratory in Cambridge (United Kingdom).

During 1916-1919, Jakub Parnas (Poles called him Jacob Karol) lived in Warsaw, where he organized a department of physiological chemistry at Warsaw University.

After the First World War, Ya. Parnassus returned to Lviv. From 1922, Yakub Parnas headed the Department of Medicinal Chemistry of the Medical Faculty of Lviv University, and from 1939 to 1941 was the Director of the Institute of Biochemistry of the Lviv Medical Institute. Before the war in 1939, he created a powerful school of biochemists in Poland. In Lviv, he organized the Institute of Medical Chemistry at Lviv University.

Yakub Parnas proved the existence of a number of re-action of intermolecular transfer of phosphate residues with the participation of adenyl nucleotides. Under his leadership, the epartment actinely participated in the study of the processes of metabolism of adenylic acid in the muscles and its role in the formation of ammonia, conducted studies of the exchange pf purine derivatives and the characteristics of their metabolism in diabetes. He developed a micromethod for determining the content in biological tissues of ammonia using a device of his own design (Parnas apparatus). Under the guidante of J. Parnas, a number of topical issues of biochemistry were studied including the exchange of carbohydtates and nucleotides. With his assistance, a biochemical laboratory and library were created at the department. Professor Parnas School has become internationally known. Jakub Karol Parnas was also known as a renowned teacher and leader of many talented biochemists. He was referred to as as a wonderful teacher who easily found common ground with a student audience.

On September 1, 1939, Poland was divided between Germany and the USSR. The Red Army entered Lviv. Parnas had a choice: he could still go to London or New York, if he would be released. Its popularity was international. He stayed in Lviv. He found it impossible to drop his students and staff, the department, the institute, which he created a factory of pharmaceuticals. He received the utmost assurances of representatives of the Soviet authorities. He stayed. He was elected to academics (and he was already a member of many academies), then he was awarded the Stalin Prize of the first degree, the Order of Lenin, the Order of the Red Banner of Labor. During the first hours of June 22, 1941, German troops moved towards Lviv. There is a legend that in order to save Parnas, at the personal instruction of Stalin, Lviv was sent to the plane of biochemistry by S.E.Manojlov and V.A. Engelhardt. This is a legend. In fact, Parnas' spouses were taken the other way – there was a car on which they arrived in Kiev. From Kiev evacuated to Ufa. And when in 1943 there was a turning point in the war, Academician J.Parnas was summoned to Moscow.

That's how I mentioned these days Parnas, being in 1945 in Lviv. " I was able to go to Moscow, but the possibility of the device seemed even more hopeless than in Ufa. I have visited several professors and one of them has made it clear to me that I will not find a place if such an order does not come from the top of power. I listened to the oppressed, but there was at least some hope of salvation. Well, he told me that in the Kremlin, near the main entrance, there is a mailbox for letters sent directly to Stalin. Such a letter must necessarily relate to the war with Germany and include specific suggestions for actions that may lead to an advantage over the enemy. I thought about it in detail and used this information. At night, he wrote a letter to Stalin, addressing the situation of Soviet soldiers on the front lines in difficult conditions of food supply, noting that there is a chemical that can survive several days of starvation and exhaustion. This substance is caffeine. Well, I suggested trying to produce caffeine from raw materials, namely chicken manure. A few days later I was offered a room suitable for a chemical laboratory, but without any equipment".

I, Parnas, settled in the privileged Metropol Hotel, where I hosted students, staff, foreign diplomats, friends and acquaintances.(Such widespread communication has changed in "known bodies," especially the special desktop, but Parnas could not have this mind). In Moscow, he became one of the founders, founded in 1943, of the Academic Medical Sciences of the USSR, the organizer and first director of the Institute of Biological and Medical Chemistry of the Academy of Medical Sciences of the USSR.

In addition, as an academic he had the right to organize his own laboratory, it was a laboratory of physiological chemistry, in which he and his closest associates continued to study carbohydrates. At the same time Jakub Karol Parnas organized a laboratory in which he directed the study of isotopes.

In 1948, the first International Biochemical Congress was held in London. J. Parnas was invited as Vice President. In this regard, he received many letter from foreign colleagues, expressing joy over the alleged possibility of communication with him. But of course, the trip to England at the time could not be a question.

Parnas was recognized as the first in the scientific hierarchy of biochemists of the USSR. The center of biochemical thought in the country was the Parnassus "Thursdays"– seminars attended by not only Muscovites but also residents of other cities.

In the field of biochemistry J. Parnas was one of the most qualified specialists: Member of the Lviv Scientific Society,

Member of the German Academy of Natural Sciences "Leopoldina," Corresponding Member of the Polish Academy of Sciences, Full Member of the USSR Academy of Sciences and Medical Academy, 19 Stalin Prize Winner Member of the Medical Academy in Paris, Honorary Doctor of Sorbonne University (Paris, France) and Athens National University (Greece).

J. Parnas had diabetes, heart failure. On October 17, 1947, he applied to be relieved of his duties as director of the Institute he had created. He would like to focus on working in the laboratory. An order to dismiss him was signed on May 28, 1948.

At the end of 1948 he was seriously ill. For the first time after his illness he was going to report to the Leningrad biochemist S. Bresler. However, he did not come to the report. Worried disciples of Jakub Karol after the meeting ran to his house and found a sad picture: his apartment was sealed, and his wife was sitting and crying in front of the door. The circumstances of his death remained unknown. He was said to have been poisoned immediately after his arrest. According to another version, he died in prison from a diabetic coma because he suffered from severe diabetes.

How did Jakub Parnas really die? At the request of Jan Jakubovych's son, Colonel of Justice V.M. Granenov wrote on July 20, 1993 (On behalf of the General Directorate for Law Enforcement in the Armed Forces) : "… On January 28, 1949, J.Parnas was arrested for carrying out intelligence activities against the USSR on the assignment of a foreign country. On the same day, he was placed in an internal prison of the MGB of the USSR, where he was examined by a doctor. The latter diagnosed with Arteriocardiosclerosis. Hypertension. Diabetes . Right inguinal hernia. In connection with the existing diseases he was prescribed treatment. January 29, 1949 at 15 h. 15 min. J.Parnas was summoned for the first interrogation by a senior investigation of the Investigative Unit on Particularly Important Cases of the Ministry of Defense of the USSR, Lietenat Colonel Ivanov. At 17.30 minutes Ivanov left Parnas in the office with the warden, and he left in connection with a job requirement. For 10-15 minutes he was told about Parnassus's poor health and a doctor's call for help. During the provision of medical care 5 pm 50 minutes J. Parnas is dead. An autopsy of J. Parnas was performed by a forensic expert who did not identify any injuries at the external examination. The death came from myocardial infarction. On April 3, 1954, a senior investigator of the KGB investigative unit of the USSR Ministry of Defense, Lieutenant Colonel Cheklin issued a resolution to suspend criminal proceedings against Parnas Jakub Karol in the absence of a crime in his actions. Documents indicating the place of burial were not preserved"

In Poland and Lviv it is mentioned. In 1986, Professor V.S. Ostrovsky published a short biography of Parnas and his memoirs about him. In 1993, a book of memoirs was published in Warsaw, edited by Irena Stasevich-Jasukova. However, just about the Soviet period of Parnassus's life there is almost nothing in these memories.

On September 9-11, 1996, a joint Ukrainian-Polish symposium was held in Lviv, dedicated to the memory of J. Parnas. The organizers- the Polish Biochemical Society, Lviv medical University, the chairman of the Organizing Committee was Professor R.S. Stoyka, Lviv.

Members of the Polish Biochemical Society arrived in Lviv. Among them was an associate of Parnassus, who worked with him until 1939, Professor Boguslaw Halikowski. The Poles brought an artistically made bronze plaque, which was solemnly installed in the lobby of the Biochemistry Department of Lviv Medical University. Headed by J.Parnas for over 20 years. At the same time, the First Parnas Conference on Biochemestry and Cell Biology was held, which initiated regular (once every 2 years) conferences. The third Parnas Conference was held in 2000 in Lviv.

Current News the year was joined by the Israel Society for Biochemistry and Molecular Biology, and the next Parnas Conference was held in 2013 in Jerusalem (Israel). The eleventh Parnassus Conference was held in 2018 in Kyiv, Ukraine. In Warsaw, the name of Jakub Parnas was named a street and is considered the founder of Polish biochemistry, and the prize of his name is the most hororable for Polish biochemists.

Bogdan Sobchuk (Fig.8) worked directly with Prof. J. Parnas in 1944 became the head of the department of biochemistry, which before the war was prof. J. Parnas headed the Lviv Medical University. Bogdan Antonovich was born on 15.03.1909 in the town of Stryi, Professor, Head of the Department of Biochemistry (1944-1973).

In 1933 he graduated from the Medical Faculty of Lviv University. In 1933-1936 he worked as an assistant at the Department of Biochemistry, Lviv University. In 1936-38 he was an assistant at the Department of Physiology of the Lviv Academy of Veterinary Medicine. In 1939-1940 he held a private practice in Ternopil. In 1940-1941 he worked as an associate professor at the department of animal husbandry, Faculty of Agriculture, Lviv Polytechnic Institute. 1942-1944 Associate Professor of the Department of Biochemistry of Medical-Natural Specialty Courses in Lviv. From 1944 to 1973 he headed the department of biochemistry of Lviv Medical Institute. I 1946 he became a candidate of medical sciences, in 1949 assistant professor of the Department of Biochemistry of the Lviv Medical Institute. In 1960 he became a doctor of biological sciences, in 1961 a professor. He is the author of 60 scientific papers, has prepared 23 candidates, 9 doctors of sciences.

Main areas of research: Carbohydrate metabolism, including the role of pyruvic acid in muscle glycogenolysis; study of the effect of xanthopterin and some by-products of its synthesis on the growth and metabolism of experimental tumors; study of iodine metabolism in the course of thyroid function in norm and pathology; study of the effect of carbon monoxide on heme proteins. On the main directions of scientific work of the Department of Biochemistry under the direction of prof. B. Sobchuk, they concerned the study of carbohydrate metabolism in malignant tumors, biochemical processes in other human pathologies, and the effects of carbon monoxide (II) on heme proteins. Prof. B. Sobchuk is a full member of the Snevchenko Scientific Society. He died on April 9, 1974 and was buried in Lviv.

In 1973, the Department of Biochemistry of the Lviv Medical Institute was headed by Mykhailo Shlemkevych, who was born on April 29 in the village Serafints of Ivano-Frankivsk region (Fig.9).

In 1950 he graduated from the Medical Faculty of Lviv Medical Institute. In 1950-1951 he worked as a radiologist in charge of the district health department of Lokach, Volyn region. For ten years in 1951-1961 he worked as a military doctor. In 1961-1964 he was the head of the department of the Lviv hospital of war invalids. During 1964-1967 he studied at the postgraduate department of the Department of Biochemistry, Lviv Medical University. In 1968 he became a candidate of medical sciences. From 1967 to 1972 he worked as an aqssistant at the same department. In 1973 he became an associate professor at the Department of Boichemistry, Lviv Medical University. The same year he headed the Biochemistry Department of Lviv Medical University until 1995. In 1986 he became a medical doctor, and in 1987 received the title of professor. He is the author of about 80 scientific and initial-methodical works, has prepared 3 PhDs.

Main direction of scientific researches: problems of endemic dental disease of the Carpathian region, experimental and clinical oncology; methodical questions of determination of carbon monoxide in air and carboxyhemoglobin in blood; found the effect of potentiation of the antitumor activity of 5-fluorouracil on the background of the introduction of ascorbic acid.

He died on November 3, 1998 and buried in Bolekhiv.

In 1995, the Department of Biochemistry was headed by Mykhaylo Tymochko, who was born on September 25, 1935 in the village of the Ivano-Frankivsk region (Fig 10).

In the 1962 he graduated from the Biological Faculty of Ivan Franko Lviv State University. In 1962-1963 he worked as a laboratory assistant at the Department of Human and Animal Physiology. In 1963-1980 he was a researcher at the Laboratory of Radiation and Physicochemical Biology, Lviv University. In 1971 he became a candidate of medical sciences. In 1978 he became a senior researcher. From 1980 to 1987 he worked as a research at the Central Research Laboratory, and from 1986-1990 – an assistant at the Department of Biochemistry, Lviv Medical Institute. In 1991 he became an associate professor of the Department of Biochemistry, in 1993 he became a doctor of biological sciences, and in 1995 he received the academic title of professor. In 1995-1998 he headed the Department of Biochemistry of Lviv Medical University.

Main direction of scientific researches: studying of biochemical and physiological mechanisms of formation of adaptation-compensatory processes under conditions of action of various exteme influences (stress, physical overload, ionizing radiation, intoxication); study of correlative relationships between intensity and efficiency of energy metabolism, oxygen-dependent reactions, and oxygen homeostasis with free-radical and antioxidant reactions; one of the authors of the discovery of the phenomenon of intracellular generation of endogenios oxygen in humans and animals.

Myknailo Tymochko is the author of about 450 scientific and teaching works, among them 10 copyright certificates for inventions, a monograph. Has prepared 8 candidates, 3 doctors of science. He died tragically as a result of an accident on July 27, 1998 and was buried at the Lychakiv Cemetery in Lviv.

In 1998 Alexander Sklyarov was elected Head of the Department of Biochemistry (Fig11).

Alexander Yakovych Sklyarov was born on October 2. 1956 in Lviv, graduated with honors from Lviv Medical Institute, studied postgraduate studies, then – assistant, associate professor of the Department of Normal Physiology. In 1983 he defended his PhD thesis, and in 1993- his PhD .

Aleksandr Yakovych is making a lot of efforts to ensure that students' education meets the modern standards of European education. With his direct participation a textbook "Clinical biochemistry " (2006), a textbook for students of higher medical institutions of the first and second levels of accreditation " Biological chemistry with biochemical research methods" (2009), a textbook for dental students "Biological Chemistry" (2015), two national (for pharmacists (2014) and medical students (2016)) textbook. The education and scientific literature for students and physicians has been supplemented by a number of manuals and reference books (17), including two editions of the manual on gastroenterology (in collaboration with Prof. E.Ya. Sklyarov and Assoc. Prof. E.R. Kosim, 1997, 2011), "Workshop on biological chemistry" (2002), "Physiology and biochemistry of digestion of animals and humans " (in collaboration with Professors V.K. Rybalchenko, T.V. Beregova et al., 2002), "Biochemical composition of body fluids and their clinical-diagnostic value" (2004), "Biochemical parameters in norm and pathology" (2007), etc.

The close contacts between the Department of Biochemistry and the scientists of Poland have a long history. During its more than 100 years of existence, the Department of Biochemistry of Lviv National Medical University has cooperated with numerous foreign scientific institutions. Until 1941, the relations between the then head of the department – Academician Jacob Parnassus and his staff with Scientists from Great Britain, Denmark, Germany, Switzerland, were intense. After the Second World War, some of the department's teachers went to Poland, but still maintains contacts with the department.

A new stage of development of close cooperation of the Department of Biochemistry with colleagues from the Department of Clinical Analytics began in 1999, when Professor Janusz Solski was in Lviv. Professor Sklyarov O.Ya. and Professor Solski J. started work on organizing multifaceted cooperation between the departments and in 1999 an agreement was signed between the departments, which was extended in 2007.

Particular attention of Professor Sklyarov O.Ya. and Professor Solsky J. devoted to the development of scientific contacts between scientists of Ukraine and Poland. This was facilitated by the organization and holding of Lviv-Lublin Conferences (Lviv-2000, 2004, Lublin -2002, 2006) on topical issues of experimental and clinical biochemistry that

occur every two years in Ukraine and Poland. Currently, the conferences bring together scholars from many cities in Ukraine and Poland and serve to develop good neighborliness between countries.

The First Lviv-Lublin Conference on Experimental and Clinical Biochemistry was held on October 12-14, 2000 in Lviv. The conference was inaugurated by its sponsors, Professor O.Ya., Head of the Department of Biochemistry of the Danylo Halytsky Lviv National Medical University. Sklyarov and Head of the Department of Clinical Analytics at the Lublin Medical Academy named after prof. Felix Skubichevsky Professor J. Solsky.

At the first section meeting "Topical problems of experimental biochemistry" was chaired by prof. O.Sklyarov and prof. V. Halyas and 20 oral reports were presented.

In 2002, the Second Lviv-Lublin Conference on Experimental and Clinical Biochemistry was held in Lublin on May 23-25. The number of participants from both Ukraine and Poland increased at this conference. The participants of the conference from different cities of Ukraine gathered in Lviv and took the bus to Lublin. The trip left many fond memories.

The section "Topical problems of clinical biochemistry," which was chaired by prof. O. Sklyarov, B. Skhidlo-Radomansky, prof. B. Lutsik, R. Matsyevsky 41 reports were presented.

Alexander Yakovych Sklyarov is known for his scientific achievements in the field of experimental gastroenterology. His scientific work is focused on the study of the mechanisms of cytoprotection and ulcerogenesis of the digestive system, diabetes, the study of the effects of gas mediators (nitrogen oxide, hydrogen sulfide), hormones, vitamins in terms of various functional states of the digestive tract and clarification "Naftusia" mineralized water in the processes of radioprotection. It was Prof. O.Ya. Sklyarov substantiated the concept of simultaneous action of neurohumoral substances in the regulation of digestive system functions; formulated the provisions of endoecology of the cavities of the digestive system; identified the metabolic processes underlying ulcerogenesis and proposed new approaches to understanding the mechanisms of cytoprotection.

He is the author of more than 450 scientific works, including 2 monographs, 10 declarative patents, 6 information sheets on new treatments and diagnostics.

The professor presents the results of his research at world European and national forums (Recoop HST Consortium, 15th, 16th United European Gastroenterology Week, 8th Meeting of France – New EU Members 16th JMRC Symposium, Lviv – Lublin conference of experimental and clinical Biochemistry, International symposium on Cell/Tissue injury and Cytoprotection/Organoprotection, Advances in pharmacology and pathology of the digestive tract), and his scientific works are published in domestic and foreign scientific publications (J. Physiol. Pharmacol., Regul. Pept., International Journal of Physiology and Pathophysiology, Stress, Digestive diseases and sciences, Current Issues in Pharmacy and Medical Sciences). He created a science school. Under his leadership 13 candidates were defended and one PhD.

Among his students are scientists, educators, doctors who work not only in Ukraine, but also abroad. Alexander Yakovych's social activity is also a multivector. He participates in the work of the specialized academic council, is a member of the editorial boards of several journals; is a member of the Physiological and Biochemical Societies of Ukraine and the World Hungarian Medical Academy (1996). O.Ya. Sklyarov- Associate Professor (1997), Academician of the Ukrainian Academy of Sciences (2004), Honored Professor of Danylo Halytsky National Medical University (2012), thanked the Mayor of Lviv for his significant contribution to the development of medicine.

## CONCLUSIONS

The development of biological research in Lviv can be divided into two major historical stages:
1) from the beginning of the founding of Lviv University in 1661 until the First World War;
2) between the First and Second World Wars and after the Second World War.

The first stage of development of biology in Lviv, in particular at Lviv University, was characterized by the dominance of research in the field of botany and zoology with the predominant use of descriptive approaches that did not require complex instruments for experimental work.

The second stage of development of biology in Lviv in the period between the First and Second World Wars was marked by a shift of emphasis in biological research from branches of science, which were dominated by observational and descriptive approaches (botany, zoology), to branches of science with extensive use of experimental, including instrumental (biochemistry, normal and pathological physiology, microbiology).

Biochemical research was initiated at the Medical Faculty of Lviv University. In 1939, the Lviv State Medical Institute was established on the basis of the medical faculty of the university, where a powerful department of biochemistry functioned, which from 1921 to 1941 was headed by a world-class biochemist – Yakub Parnas.

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REVIEW ARTICLE

# DEVELOPMENT OF INCLUSIVE EDUCATION IN UKRAINE IN THE CONTEXT OF WORLD TRENDS

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**ABSTRACT**
The aim of the research is to analyze the problem of inclusive education in Ukraine in the context of world trends.
According to its philosophy, each school should be inclusive, which implies the readiness of the school to accept every child with SEN at any time, the desire to create the most favorable educational environment that will serve the development of its potential. According to the results of the analysis, we also conclude that Ukrainian scientists pay more attention to issues of inclusion related to higher education (foreign experience, features of teaching students with inclusion, training future teachers to work with children with disabilities, etc.). Meanwhile, in our opinion, the theoretical and methodological principles, pedagogical conditions, practical methods of organizing inclusive education of children with various types of developmental disabilities are insufficiently studied. This, among other things, highlights the scientific problem raised in our study.
Thus, the attention of the Ukrainian state to people with SEN, European-oriented social educational policy is evidenced not only by the reduction of the number of boarding schools, orphans and children deprived of parental care there, but also the dynamics of growing enrollment of children with SEN in inclusive special classes of secondary schools (in 2020/2021 academic year compared to 2015/2016 academic year this amount increased by 7% (from 5.3 thousand to 5.7 thousand). As a result of the development of inclusive education over the past five years, the number of students with SEN covered by inclusive education has increased more than 6 times, and the number of inclusive classes in secondary schools has increased 7 times. Similar changes have taken place in the staffing of inclusive education. Inclusive education and upbringing of children with SEN is a long-term strategy that is considered not as a local area of work, but as a systematic approach to organizing the activities of the general education system in all areas in general.

 **KEY WORDS:** inclusive education, children with special educational needs, National Strategy for inclusive education development, inclusive classes

## INTRODUCTION

The modern system of school education is focused on a humanistic approach to the child as a developing person who needs an understanding of his interests and rights as well as respect for them. The idea of creating optimal conditions for the development of the personality of a student with special educational needs (hereinafter – SEN), the formation of his activity comes to the fore. A primary school student with SEN should feel like an active participant in the educational process, the task of which is to help creating conditions that open the child to independent action to learn about the world around him, get an education, develop creative abilities, socialize, prepare for life and further study. The draft of the National Strategy for the Development of Inclusive Education for the period 2020-2030 identifies new conceptual approaches to the education of children with disabilities, implementation of ideas of integrated education and upbringing of children with SEN with normally developing peers [1-8]. In this

regard, ensuring the realization of the right of children with disabilities to education is considered one of the most important tasks of state policy of Ukraine not only in the field of education, but also in the field of demographic and socio-economic development.

Ukrainian and foreign scholars are actively researching various aspects of the organization and functioning of inclusive education environment (hereinafter IEE), features of inclusive education, substantiate the conceptual foundations of teaching students with SEN in an inclusive school environment; scientists analyze the creation of various conceptual and structural models of IEE; consider the problems of interaction of psychological and pedagogical specialists within the IEE, etc.

## THE AIM

The aim of the research is to analyze the problem of inclusive education in Ukraine in the context of world trends.
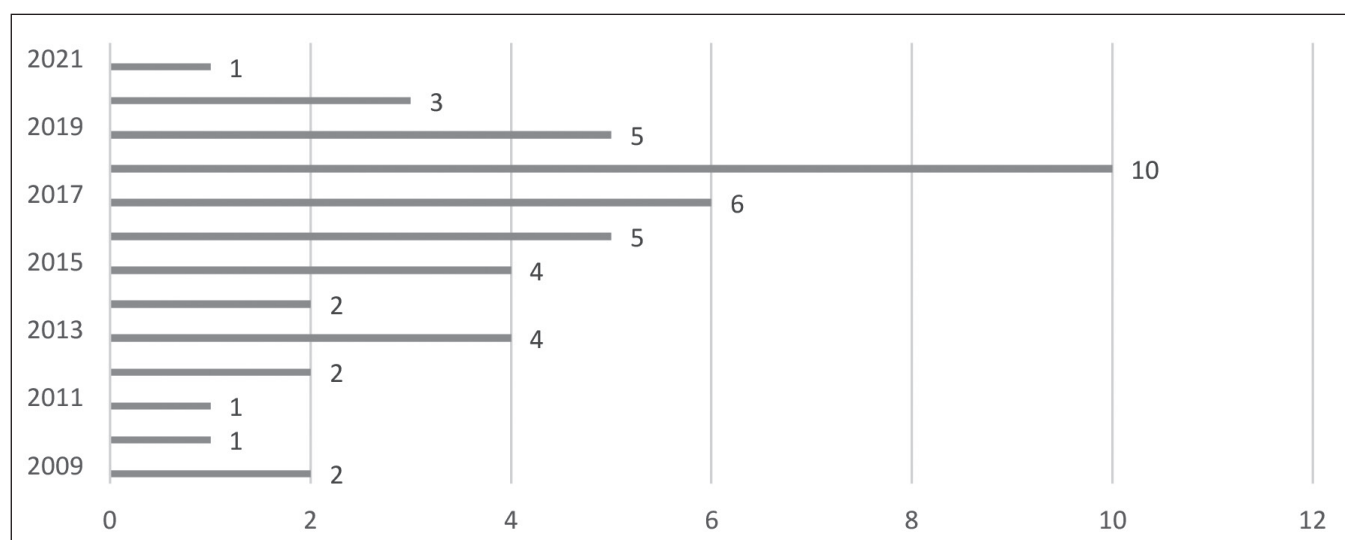
**Fig. 1.** Letters, orders of the Ministry of Education and Science of Ukraine, which regulate the education of children with SEN (2009-2021)

## REVIEW

According to its philosophy, each school should be inclusive, which implies the readiness of the school to accept every child with SEN at any time, the desire to create the most favorable educational environment that will serve the development of its potential [5; 7]. According to the Ministry of Education and Science of Ukraine, expenditures on state support for children with SEN are increasing, for example, in 2017, according to the Law of Ukraine "On the State Budget of Ukraine for 2017", the state allocated 209458.3 thousand hryvnias for persons with special educational needs [5; 7]. This testifies to the attention of our country and the real steps of the Ministry of Education and Science of Ukraine to people with SEN.

According to the results of our analysis of the approved letters, orders, positions of the legislature on the organization of education, guardianship, support of children with SEN during 2009-2021 [5; 7] we concluded that the appropriate legal basis for the education of this category of children (Fig. 1 highlights the dynamics of the legislative provision of education for people with SEN).

The reduction of the network of boarding schools, orphans and children deprived of parental care also testifies to the European-oriented social educational policy of Ukraine. During 2011-2017, the network of boarding schools for this category of children decreased significantly, for example, the number of orphanages decreased by 67% and boarding schools for children – by 86%. 2016/2017 (compared to 2015/2016 academic year) the number of special secondary schools and the number of their students decreased by 5.1% (from 32.6 thousand to 31.7 thousand) [5; 7]. This trend continues nowadays. Due to various reasons, the network of training and rehabilitation centers, i.e. educational institutions for children with severe developmental disabilities, as well as their students, increased by 22.4% during this period. The same dynamics can be observed with regard to the coverage of children with SEN with education in inclusive special classes of secondary schools (Table I). Thus, the number of students with SEN

for whom the educational process is organized in special classes of secondary schools (at the place of residence) increased by 7% (from 5.3 thousand to 5.7 thousand).

According to official data, in Ukraine there were 635 inclusive resource centers, 43% of schools had inclusive classes (18,687 classes) with 25,078 students in 2020. Unobstructed access was provided for people with SEN in 11,775 general secondary education institutions. The number of teacher assistant rates had been increased to 17,215 assistants [7]. According to official data, presented in table 1, as a result of the development of inclusive education over the past five years, the number of students with SEN covered by inclusive education has increased more than 6 times, and the number of inclusive classes in secondary schools has increased 7 times. The same qualitative and quantitative changes have taken place in the staffing of inclusive education: if in 2016/2017 academic year 1 825 assistants worked with children with SEN, then 2020/2021 this number reached the mark of 17,215 [7].

Thus, in Ukraine, as well as in the world in general, we see a steady optimization of institutional education, which is primarily due to the introduction of inclusive education and an increase in the number of students with SEN enrolled in special classes of general secondary education. State educational policy, Ukraine's course towards European integration as well as normative and legal changes serve to form a new philosophy in Ukrainian society regarding students with SEN. However, in some regions of Ukraine there are different rates of development of inclusive education (Fig. 2). As you can see, in Ivano-Frankivsk, Kharkiv, Mykolayiv the rate of inclusion of inclusive education for students differs from the national average (Fig. 2), they are quite low [5; 7].

Approximately the same situation is in the number of inclusive, special classes and their children in terms of different regions as of 2016/2017. In Ivano-Frankivsk region, for example, there is a tendency to increase such classes, but the dynamics compared to some regions of
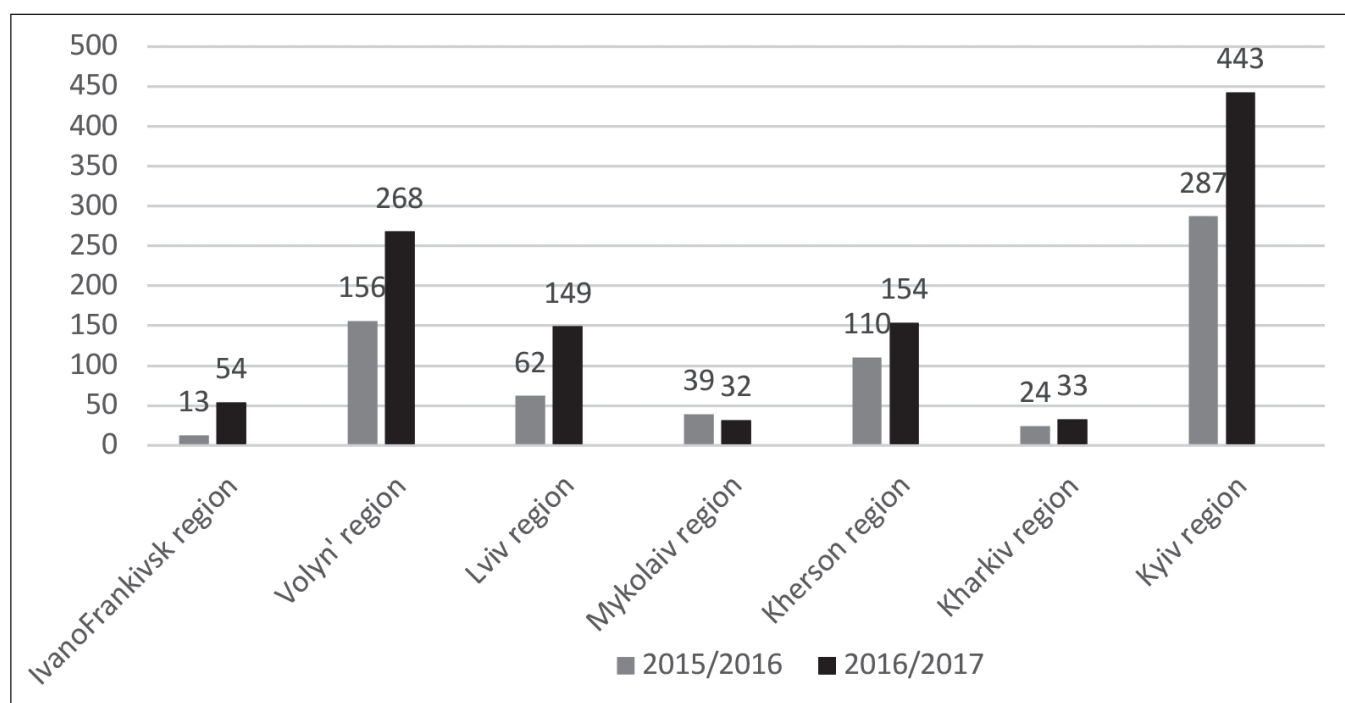
**Fig. 2.** Coverage of inclusive education of students with SEN in some regions of Ukraine
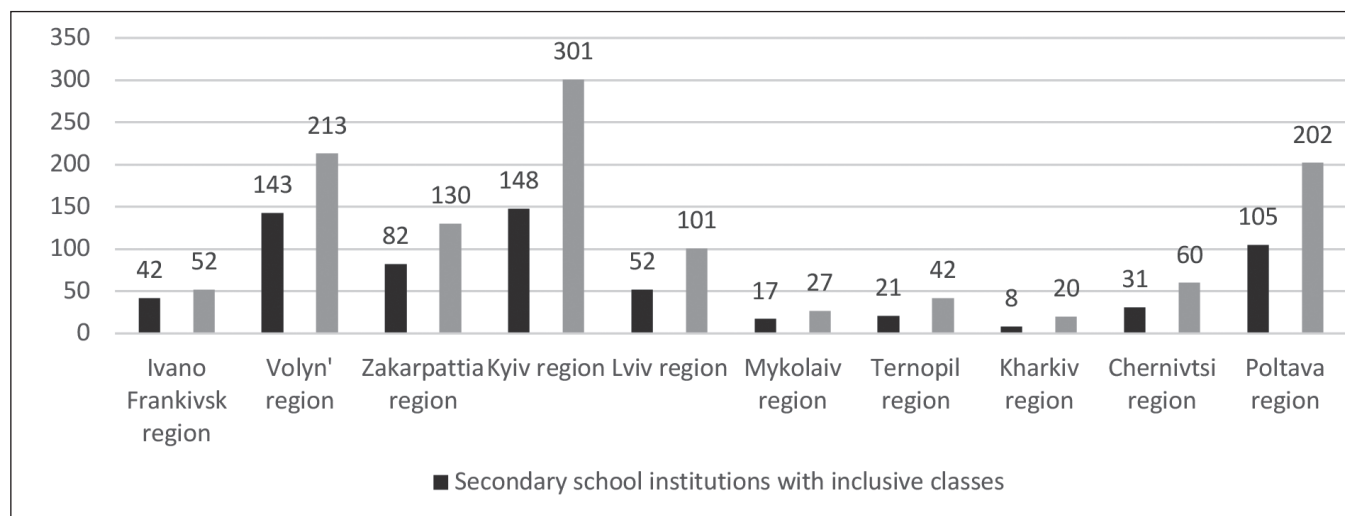


**Fig. 3.** The number of inclusive, special classes and their children in some regions of Ukraine as of 2016/2017.

Ukraine is not so vivid, in other words, these rates are quite slow (Fig. 3).

Despite the fact that Ukraine has made a strong step forward in the establishment, development and functioning of inclusive education, despite numerous changes in legislation and regulations to support inclusive education for children with disabilities, the real spread of inclusive education in the country is somewhat low, as in 2015/2016 academic year more than 50,000 children with SEN were out of quality education and socialization [8].

It is important to note that inclusive education in Ukraine, as in the whole civilized world, is rightly called a humane alternative to the boarding education system, where children with SEN study in special educational institutions, forced to live in boarding schools because of their

territorial diversification. Despite the fact that a proper inclusive educational environment has been created here, children with SEN are deprived of the conditions, ways and means for joint learning, education and development, taking into account their needs and opportunities, which can be offered by a secondary school that removes barriers in the system of education and support system for students with SEN, provides opportunities for socialization, development of natural abilities, interests and serves for further integration into society, opens opportunities for admission to vocational and higher education institutions, allows to establish friendly relations with peers, students and beyond its borders, to model effective ways of interaction with class and school collective, etc. that is the most important for such people [2; 4].

**Table I.** Data on children with SEN in inclusive classes of secondary schools (comparison as of 2015/2016, 2016/2017, 2020/2021 academic years] [5; 7]

| Academic year | Number of inclusive classes at the secondary school institutions | Number of students with SEN who attend secondary school institutions |
|---|---|---|
| 2015/2016 | **1 518** | 2 720 |
| 2016/2017 | 2 715 | 4 180 |
| 2020/2021 | 18 681 | 25 078 |

**Table II.** Advantages and problems associated with the introduction of inclusive education in secondary school

| Advantages | Problems |
|---|---|
| increasing the level of social interaction with their healthy peers [1; 2; 3; 4; 6; 21] | the existing language and speech difficulties of children with SEN lead to bullying at school by British scientists, resulting in low self-esteem among students (British scientists Jeff Lindsay, Julie E. Dockrell and Claire McKee [16]) |
| social acceptance of children with special needs and friendship between children [1; 2; 3; 4; 6; 10; 14; 21] | rejection of children with SEN and bullying, which negatively affects their socio-emotional development (British scientists Elian Fink, Jessica Dayton, Neil Humphrey and Miranda Walpert) [12], S. J. Freeman and M.S. Alkin [13]) |
| improving social competence [1; 2; 3; 10; 14; 21] | many children are only now gaining their first social experience, which creates additional problems in the adaptation of children, building long-term relationships with peers [4; 6] |
| improving academic achievement [1; 3; 4; 6; 21; 24] | possible isolation of children with SEN within the class, group, school (Greek researchers (Eleni Didascalou, Anastasia, Eleni Andreu) [11]), English scientists W. Stevens, I. de Bourdougou, P. Van Oost [22]) |
| improving communication skills [2; 6; 24] | students with SEN are often dissatisfied with joint learning with healthy children, they are upset by the negative attitude of classmates (Elena Didaskalou, Anastasia, Elena Andreu [11]) |
| expanding opportunities for communication and play with more competent social partners (Elian Fink, Jessica Daytona, Neil Humphrey and Miranda Walpert [12]) | difficulties in communicating with teachers (British scientists Elian Fink, Jessica Dayton, Neil Humphrey and Miranda Walpert) [12], S. J. Freeman and MS Alkin [13]) |
| increasing the degree of participation of each student in the academic and social life of the school, as well as reducing the degree of isolation of students in all processes occurring within the school [1; 3; 4; 6; 21] | unwillingness of parents of both healthy children and children with disabilities to send their child to an inclusive school (British scientists Elian Fink, Jessica Dayton, Neil Humphrey and Miranda Walpert) [12], S. J. Freeman and MS Alkin [13] ) |
| | the complexity of the process of social interactions with peers in children with SEN (K. Salmivalli [20], W. Stevens, I. De Bourdougou, P. Van Oost [22]) |
| | lower social status of students with SEN compared to healthy peers (W. Stevens, I. de Bourdougou, P. Van Oost [22]) |
| | children with SEN are often perceived by peers as lacking communicative competence (American scientists Kuhne M. & Wiener J., Lindsay G., Dockrell J. E. & Mackie C. [15], etc.]). |
| | capricious behavior of children with autism spectrum disorders and their landing in the "healthy class" provokes a negative attitude of peers; children with mental retardation and mental retardation often cause intolerance of healthy children, cases of bullying [11; 12; 15; 17]. |
| | lack of experience of friendship, close, mutual, dyadic relations (Catherine L. Bagwell and Michelle E. Schmidt [9], D. K. Lipsky and Gartner A. [16], American scientists (K. H. Rubin, W. Bukowski & J. Parker) |

The results of the analysis of the updated source base testify to the great attention of foreign [9-24] and national scientists [1-4; 6; 21] to the problems of inclusive education, which cover various issues related to the peculiarities of child development, special correctional education, organization and functioning of inclusive education,

training to work with children with SEN, the activities of a teacher's assistant in an inclusive classroom, etc. Scientists as representatives of various fields of pedagogical knowledge and education recognized the need for inclusive education, and some of them, such as O. Andreeva, V. Bekh, V. Sukhomlinsky, M. Yarmachenko and others

actualized the problem of inclusion, laid the foundations of open learning and education in a social environment in Ukrainian pedagogical science. Psychological, legal, social, pedagogical aspects of the issue are also deeply researched by foreign English-speaking scholars (F. Armstrong, M. Davis, J. Bellow, K. Jenks, X. Kerbo, S. Corlett, M. Crozier, F. Crosby, D. Cooper, T. Newman, G. Silver, P. Silver, W. Schmidt, K. Taylor, A. Hodkinson, etc.). According to the results of the analysis, we also conclude that Ukrainian scientists pay more attention to issues of inclusion related to higher education (foreign experience, features of teaching students with inclusion, training future teachers to work with children with disabilities, etc.).

Meanwhile, in our opinion, the theoretical and methodological principles, pedagogical conditions, practical methods of organizing inclusive education of children with various types of developmental disabilities are insufficiently studied. This, among other things, highlights the scientific problem raised in our study.

## DISCUSSION

Based on the above, we draw intermediate conclusions that the theory and practice of inclusive education of children with SEN is becoming increasingly common in Ukrainian pedagogical and psychological science. Organizing inclusive education, educators actively use these developments. The education system is gradually being restructured in line with global trends, however, society is not always ready for such changes. This causes conflicts of interest between the subjects of the educational system. Moreover, as educators who work with children with SEN in primary school, we are witnessing contradictions that arise within the school system itself, when parents are often focused on achieving high results in their child's learning, the intensity of the educational process and often do not understand the obstacles, due to the peculiarities of the physical or mental development of their child are on this path to success. Thus, the demand of the state, the educational system, educational institutions and teachers is faced with the unwillingness of society to accept it. The most difficult, however, is the problem of children's perception of a child with SEN. This is evidenced not only by the results of studying the sources of the problem, but also their own observations of the pedagogical process. In schools of the Ivano-Frankivsk region, the attitude towards them is tolerant, but sometimes it was necessary to trace the unfriendly attitude of peers to children with intellectual disabilities. This is a big problem, because under such conditions, a student with SEN feels like an outcast. Thus, we agree with the opinion of a number of Ukrainian and foreign scholars that the very idea of inclusive education is to ensure not only equal access to educational services, but also equal treatment of children regardless of their characteristics. The formation of a positive attitude towards children with SEN contributes not only to their successful integration into the team of peers, but also to business interaction within the educational process, without which full-fledged learning

in the classroom is impossible. In our opinion, the leading role in this process should be given to communicative activity, because we consider the communicative interaction of primary school students as a tool for forming a positive attitude of students to children with SEN.

We update the achievements of foreign scientists in this context to highlight the problem of forming communication skills of students with SEN in the global dimension.

According to the results of the analysis of English-language sources available on the Internet, scientists from Europe, both American continents, the East, i.e. from all scientific centers around the world raise various aspects of the problem of inclusive and integrated education of children with SEN.

First of all, researchers note that the possibility of education is of great importance for people with SEN, as it allows them not only to discover their potential, but also to be realized in life, to integrate into work and society [1; 3; 4; 6]. The inclusive process in education is understood by scientists as a specially organized educational process that ensures the inclusion and acceptance of a child with disabilities in the environment of ordinary peers. This means that inclusive education is based on the important premise that all children, regardless of physical, mental, intellectual and other characteristics, should be included in the general education system, they study and are brought up together with their peers at the place of residence in general education, which takes into account their special educational needs and provides the necessary special support, where the characteristics of these students are treated with understanding, positive and perceive individual characteristics not as a problem but as an opportunity to enrich the learning process.

According to the results of researches of foreign and Ukrainian scientists, we have singled out the actualized advantages of inclusive education, they are expressed in table 2, immediately noted the problems caused by inclusive education, we will comment on them below.

According to the analysis of the source base, for example, the work of individual teachers from the United States (Xin Zhang, Richard S. Anderson, Ji Jung Lin, Joshua Morris [23], Great Britain (Jeff Lindsay, Julie E. Dockrell and Claire McKee [15]) etc. agree that the world pedagogical community emphasizes the importance of communicative interaction in teaching students with SEN, highlight the communicative-activity approach in the educational process and inclusive classroom, promotes teaching based on discussion of certain issues, as it creates opportunities for students to practice important skills such as argumentation, critical thinking, cooperation, etc. We completely agree with the opinion of authoritative foreign scholars (Martin Nystrand [18], Catherine O'Connor, Suzanne Chapin and Sarah Michaels [19]; etc.) that communicative interaction allows students to express and defend their views, work in research communities, share their own opinions with their peers, as well as on the basis of communicative interaction, students with SEN develop thinking skills, form a better understanding of the material which they have read and

passed, increase children's involvement in the learning process and acquire skills of cooperation and communication. Our observations of students' with SEN learning are confirmed by the results of research by Margaret J. Mc Caune, Isabel L. Beck and Ronett GK Blake in the area of communicative interaction of children with various disabilities. Thus, among the educational tasks of this process, scientists mention the following ones: students learn to understand and appreciate different points of view, develop the ability to analyze a wide range of issues, formulate their own positions on these issues, and propose and defend arguments of their own position [17].

The study of the updated sources gives grounds for the following conclusion. In our opinion, scientists did not pay enough attention to the role of play in the development of communication skills of primary school students with SEN, cooperation and interaction of classmates and children with SEN in extracurricular activities. That is also essential as the primary school age is characterized by high importance for children of the educational process and the authoritative figure of the teacher. In our opinion, the organization of communicative interaction should begin from the very beginning of school life of an inclusive class and be realized within the educational process, not only by means of a lesson, but also on breaks, in extracurricular activities. The leading form should be not only educational activities, but also games.

Under such conditions, the development of students' with SEN communication skills will play a key role in developing relationships with people around this category of children, and thus determine the success of building a life path in the process of not only learning but also later life. Early school age is a favorable period for the formation of communication skills. The high degree of children's focus on learning activities gives teachers the opportunity to use the lesson to apply the practice of communicative interaction in an inclusive classroom.

The National Strategy for the Development of Inclusive Education for 2020–2030 [6] identifies special conditions that need to be created for the successful education (upbringing) of children and adolescents with disabilities. In particular, these are special educational programs and teaching methods, textbooks, manuals, didactic and visual materials, technical means of teaching collective and individual use (including special), means of communication, sign language translation in the implementation of educational programs, as well as pedagogical, psychological and pedagogical, medical, social and other services that provide a barrier-free environment and living, without which the development of educational programs for people with disabilities is impossible (or difficult at least) [4; 6].

Nowadays, among the problems of inclusive education in Ukraine there is an issue of staffing, as well as the construction of a holistic system of training, retraining and advanced training of specialists based on the best international and Ukrainian experience; maximum use of opportunities of educational, scientific, public organizations, system of private business education, active and

interactive technologies of training, internships in Ukraine and abroad; creation of a modern system of attestation of specialists and assignment of qualification categories, use of modern motivational technologies, effective forms of remuneration, etc.

After analyzing the sources and making our own observations of the educational process for children with SEN in secondary school, the practice of inclusive education in rural areas, a number of problems, difficulties, we have systematized some obstacles in the development of inclusive education. Here are some of them: spontaneous integration into a secondary school, unwillingness of teachers to work with any child with SEN; for most educational institutions, the problem of inclusion is, unfortunately, not relevant (this leads to the fact that existing institutions do not cope with the growing demand of the population and tend towards the appropriate correctional educational institution); insufficient material and technical base for children with SEN, lack of necessary premises, means of education and leisure; non-elaboration of normative documents both at the level of educational programs and at the level of documents that regulate the financial and legal support of the educational process, including the transition from system to system, from level to level; the National Strategy for the Development of Inclusive Education for 2020–2030 needs to be adopted; the high academic requirements offered to students with SEN in the Ukrainian educational system cause problems with adapting the high school curriculum to the needs of a child with special needs; insufficient provision of relevant employees of secondary schools with inclusive classes, lack of proper training of the entire teaching staff; due attention is not always paid by the higher school to the pedagogical teams of the secondary school with inclusive classes, for the most part higher education is discrete and does not meet the needs of practice; insufficient disciplines are introduced into the system of teacher training of mass secondary schools, which contribute to the understanding of future teachers of different levels of children's readiness for learning, the specifics of working with students with SEN; there is no qualitative complex diagnostics of the child with SEN; here is no adequate number of highly professional specialists (special educators, speech therapists, social educators, rehabilitation specialists, etc.) to work with children with SEN, especially in rural schools; the individual trajectory of education of a child with SEN is not always clearly developed; insufficient activity of the resource center of inclusive education at united territorial community; weak involvement of the potential of specialists of correctional schools, practically no interaction between specialists of regular and correctional institutions, and this would contribute to the best possible understanding of the problems of deviations in child development; it is necessary to involve the methodological potential of consultants in the psychological, medical and educational commissions, employees of inclusive resource centers, as the psychological center cannot be a full-fledged resource

for school educational institutions in preparation of methodical support for subject teaching; the development of the volunteer movement in educational institutions for students with SEN special updating; the development of the parent movement can help cooperation programs, parents and schools, the result of the activity of parents, school, resource center, volunteers can be positive partnership courses and a forum for parents; the continuity of primary and secondary education (as well as higher) for children with SEN is insufficiently taken into account, in the process of transition to secondary school the problems of inclusive education only become more acute and complicated, in particular much more rigid requirements for educational programs and their results, etc.

## CONCLUSIONS

Thus, the attention of the Ukrainian state to people with SEN, European-oriented social educational policy is evidenced not only by the reduction of the number of boarding schools, orphans and children deprived of parental care there, but also the dynamics of growing enrollment of children with SEN in inclusive special classes of secondary schools (in 2020/2021 academic year compared to 2015/2016 academic year this amount increased by 7% (from 5.3 thousand to 5.7 thousand). The number of students with special needs for whom the educational process organized in special classes of secondary schools at the place of their residence was also increased. As a result of the development of inclusive education over the past five years, the number of students with SEN covered by inclusive education has increased more than 6 times, and the number of inclusive classes in secondary schools has increased 7 times. Similar changes have taken place in the staffing of inclusive education. Inclusive education and upbringing of children with SEN is a long-term strategy that is considered not as a local area of work, but as a systematic approach to organizing the activities of the general education system in all areas in general.

Having analyzed the sources and own observations of the educational process of students with SEN in primary school, the practice of inclusive education in rural areas we singled out a number of problems in the development of inclusive education, including spontaneous integration into secondary school, unpreparedness teachers to work with any child with SEN; the problem of inclusion which is unfortunately not relevant for most educational institutions. This leads to the fact that existing institutions do not cope with the growing demand of the population and tend towards the appropriate correctional educational institution; insufficient material and technical base for children with SEN, lack of necessary premises, means of education and leisure; non-elaboration of normative documents both at the level of educational programs and at the level of documents that regulate the financial and legal support of the educational process, including the transition from system to system, from level to level; high academic requirements offered by the Ukrainian educational system as

they cause problems with the adaptation of the high school program to the needs of a child with special needs. It also greatly hinders the introduction of foreign experience in Ukrainian secondary schools (for example, basic education in the UK is more focused on practical knowledge of the world, there is a high status of training in colleges, which simplifies the task of inclusion); the emphasis of the school and parents on the higher education of children. However, real research shows that the academic nature of the Ukrainian educational program is under the power of only 15-20% of students, all others are under stress of unbearable workload and grow up with complexes of non-compliance with the requirements of the world; this is especially felt by students with SEN. The development of inclusive education in Ukraine is directly dependent on the solution of financing the stay of students with SEN in a regular school (a child in a special correctional institution is assigned a larger amount of funds than the same student in a secondary school), the similar situation is observed in the rates of the staff list of pedagogical workers; insufficient qualification of staff capable of working in an inclusive classroom; insufficient provision of relevant employees of secondary schools with inclusive classes, lack of proper training of the entire teaching staff; not given due attention by the higher school to the pedagogical teams of the secondary school with inclusive classes, for the most part higher education is discrete, does not meet the needs of practice. Insufficient disciplines are introduced into the system of teacher training of mass secondary schools, which contribute to the understanding of future teachers of different levels of children's readiness for learning, the specifics of working with students with SEN; there is no qualitative complex diagnostics of the child with SEN; there is no adequate number of highly professional specialists (special educators, speech therapists, social educators, rehabilitation specialists, etc.) to work with children with SEN; there is no individual trajectory of education of a child with SEN; insufficient activity of the resource center of inclusive education at the united territorial communities; the potential of correctional school specialists is weakly involved, there is practically no interaction between specialists of regular and correctional institutions, and this would contribute to the best possible understanding of the problems of deviations in child development; it is necessary to involve the methodological potential of consultants of psychological, medical and pedagogical commissions (PMPC), employees of inclusive resource centers, as the psychological center cannot be a full-fledged resource for secondary schools in the preparation of methodological support for subject teaching; the development of the volunteer movement in educational institutions for students with SEN special updating; the development of the parent movement can help cooperation programs, parents and schools, the result of the activity of parents, school, resource center, educational scientific centers and volunteers can be positive partnership courses and a forum for parents; the continuity of primary and secondary education (as well as higher) for children with SEN is insufficiently taken into account, in the process

of transition to secondary school the problems of inclusive education only become more acute and complicated, in particular much more rigid (both in public consciousness and in normative-documentary design) requirements for educational programs and their results, etc.

The basic principles of development of communicative skills of students with SEN, which are presented in modern psychological and pedagogical literature, the analysis of works of Ukrainian and foreign authors in the field of inclusive education and upbringing of a child with SEN, as well as current international and domestic government documents regulate the development of inclusive education. The analysis of the actualized source base, including English-language publications, works of M. Montessori and other scientists who studied the development of children with SEN, allowed us to conclude that communicative awareness, speech activity is the most important means of communication, without which the existence and development of human society, education of the child, and the formation of speech skills as a component of communicative development of students with SEN is an urgent problem today, the solution of which is of great importance for each child with disabilities, and for society as a whole. It is proved that the communicative skills of primary school students with SEN play an important role in the process of personal development of human values, knowledge, self-realization as a creative, unique, tolerant personality in the course of socialization and social interaction in inclusive education.

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CASE STUDY

# VALUE OF ULTRASONOGRAPHY METHOD IN THE DIAGNOSIS OF TEMPOROMANDIBULAR DISORDERS AND PATIENT MANAGEMENT MONITORING. CASE REPORT

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**ABSTRACT**
Value of ultrasonography method in the diagnosis of temporomandibular disorders and patient management monitoring is underestimated. Application of ultrasonography in the diagnosis of patients with temporomandibular disorders in many countries is limited. The main advantage and feature of the method is the ability to conduct examination in dynamics (during the function). Its safety and availability allow applying the method often and at different stages of diagnosis and treatment. So, ultrasonography fully satisfies the needs of primary diagnosing. MRI remains the recognized «gold standard» for the diagnosis of temporomandibular disorders.
A case report which is presented, demonstrates the relevance and scope of diagnostic information obtained by ultrasonography, and their verification and clarification with the use of magnetic resonance imaging.
Upon comparing the results of USG and MRI described in the clinical case report, we can conclude that ultrasound is quite sensitive and specific in diagnosing anterior disc displacement and blocked movement of the head of the mandible.

**KEY WORDS:** Temporomandibular Disorders, Temporomandibular Joint, Diagnosis, Ultrasonography, Magnetic resonance imaging

## INTRODUCTION

Temporomandibular disorders have gained an increasing attention in modern society, with the development of additional methods of diagnosis and digitalization. This problem is studied intensively in all civilized countries. There is no unanimous opinion on the etiology and pathogenesis yet. It is unambiguously clear that such pathology lies at the intersection of specialties and is actively studied in dentistry, neurology, physiotherapy, psychotherapy, rheumatology etc.

At the same time, there is a problem of clear differential diagnosis in order to determine treatment strategy and monitoring with evaluation of long-term results. Comprehensive differential diagnosis of TMD is possible by conducting general clinical and special methods of investigation. The ability to correctly interpret the diagnostic information received takes on particular importance [1-3]. During the examination and diagnosis of temporomandibular disorder, it is necessary to follow the procedure of examination protocols for the patients with suspected TMD [4]. The search for examination algorithms, establishing diagnosis, clear classification and nosological forms, and, accordingly, treatment protocols remain the most discussed topics among researchers of this speciality.

According to various data, the incidence of temporomandibular disorders among population ranges from 12 to 85% [5]. Females of reproductive age, urban dwellers, are affected more often. Temporomandibular disorders are not a monopathy, and require a comprehensive clinical examination by various specialists with expanded conclusions, as well as specialized additional diagnostics. It is known that the evidence of temporomandibular disorders is often found in other diseases of both dental and general somatic origin. More than 30 diseases have already been identified, the symptoms of which may mimic temporomandibular disorders. Physicians looking for etiological factors in the development of such disorders should be knowledgeable about information from different fields of medicine, and be able to pool and analyze it. As practice shows, it turned out to be more difficult than expected [6].

The complexity of the situation is also associated with temporomandibular joints structure peculiarities, the coherence between masticatory muscles, general physical and emotional state of a patient, the presence of concomitant somatic pathologies. Sometimes, it is difficult to reveal this pathology at an early stage. This results in complications and chronicity of the process. Consequently, treatment is often ineffective.

The key role in the assessment of TMJ is played by radiological methods of examination: X-ray study, cone beam computed tomography (CBCT), magnetic resonance imaging (MRI), ultrasonography (USG). Each of these methods addresses certain tasks. Traditional X-ray study, orthopantomography (OPG) and zonography (ZG) allow

**Table I.** Informative value of different study methods, depending on hard or soft tissues of the joint involvement [2].

| TMJ structures | Method of study and its informative value | | | | |
|---|---|---|---|---|---|
| | OPG | ZG | CBCT | USG | MRI |
| Hard tissues | | | | | |
| Bone ankylosis | + | + | +++ | - | + |
| Arthritis/ Arthrosis | + | + | ++ | ++ | +++ |
| Developmental disorders | ++ | ++ | +++ | - | + |
| Neoplasms | + | ++ | +++ | + | +++ |
| Fractures | ++ | ++ | +++ | - | ++ |
| Soft tissues | | | | | |
| Intraarticular disorders (displacement of articular disc) | - | - | - | ++ | +++ |
| Disc perforation | - | - | - | ++ | +++ |
| Fibrous ankylosis | - | - | - | ++ | +++ |
| Exudation | - | - | | ++ | +++ |
| Joint ligaments, muscles | - | - | - | ++ | +++ |
| Articular capsule inflammation | - | - | - | ++ | ++ |

(- method is not used, + method is rarely used, ++ method is often used, +++ the leading method of study of the structure specified)

obtaining information on the bone structures of the joint, assessing the joint space. CBCT method can be used to obtain TMJ images in different planes and to assess the bone structures of the joint. Assessment of soft tissue structures using these methods is impossible [7]. Assessment of articular disc, ligaments, muscle structures can be obtained by USG and MRI [8].

The results of works concerned with ultrasonographic method of TMJ examination have been published in the literature [7-19]. The comparative analysis of ultrasonographic method of imaging with other methods of TMJ diagnostics, first of all MRI is presented. MRI is described in the literature as the "gold standard" [13-17]. Those advantages: time, efficiency, cost-effectiveness cause USG to be an alternative method of diagnostics [18].

Therefore, the coordinated cooperation of dentists with a number of other specialists in finding etiological factors and prescribing the correct management remains relevant. The choice of appropriate method of diagnostics is the key to success in establishing a diagnosis and early treatment. Therefore, understanding the procedure of USG and MRI allows a dentist to correctly interpret the description of the results, and use them to the benefit of the patient.

## CASE REPORT

Patient P., born in 1976 (41 years old). Complained of pain in the left parotid region at rest and during chewing, which lasted for about a month. Life history included craniocerebral injury of the frontal area on the right (road traffic accident 10 years ago), listhesis in the cervical spine, multinodular goiter (undergoes follow-up examinations and treatment by endocrinologist), the initial signs of osteoarthritis of both knees, hypotension, migraine. Results

of clinical examination revealed minor facial asymmetry due to the reduced left side, reduced tone of masticatory muscles, mouth opening 38 mm, its trajectory is sharp deflection to the left, absent acoustic phenomena in the joints, pain in the left parotid region at maximal opening of mouth and protrusion, positive response on the left to the provocation compression test of TMJ. Intraoral examination: mucous membrane and periodontal tissues are unremarkable, class III defects of dentition of the mandible, subclass 1 by Kennedy, missing teeth 36 and 46. Occlusion: signs of orthognathic occlusion were found in the frontal region, teeth 16 and 26 in the lateral regions are below the occlusal plane due to the protrusion towards the defects of dentition of the mandible. Among additional examinations, the patient has two orthopantomograms: current one (Figure 1) and the one made 6 years ago (Figure 2), and ultrasonographic study of TMJ and masticatory muscles.

USG findings: anterior displacement of articular disc of the left TMJ with blocked movement of the head of mandible (without reduction). Initial degenerative changes of the head of the left TMJ. Due to the fact that USG was conducted in dynamics, and interpretation of the image for the dentist was complicated, proceeding from the extensive experience of the doctor, we give findings without the image. To specify the diagnosis and etiology of the disease, MRI of TMJ with closed and open mouth position, as well as dynamic MRI were prescribed (Figure 3).

MRI findings: anterior disc displacement in the left TMJ; secondary manifestations of osteoarthritis of the left TMJ with joint effusion, reactive changes of the left lateral pterygoid muscle.

Detailed comparison of the results of clinical examination with the results of follow-up surveys revealed signs of deforming arthrosis of the left TMJ in the acute stage.
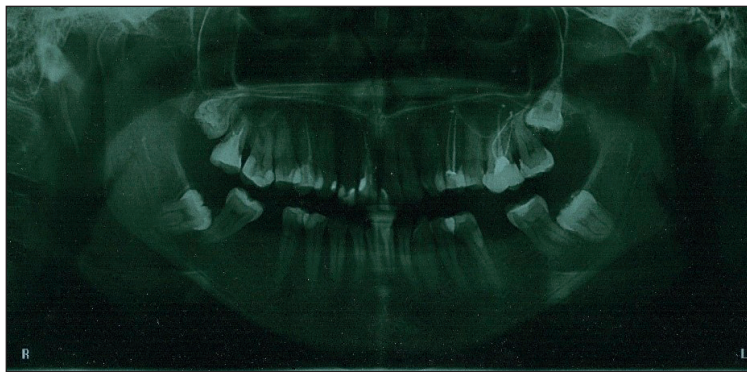
901

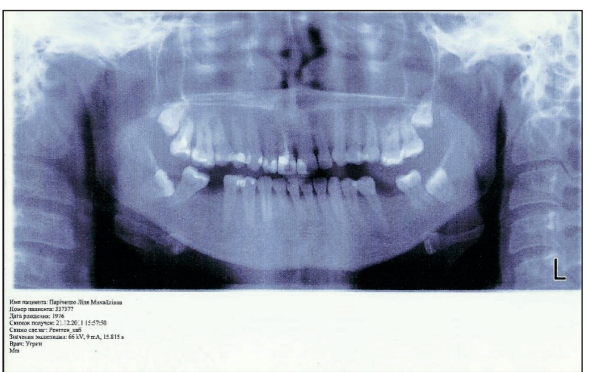**Fig. 1.** Diagnostic orthopantomogram (2017)



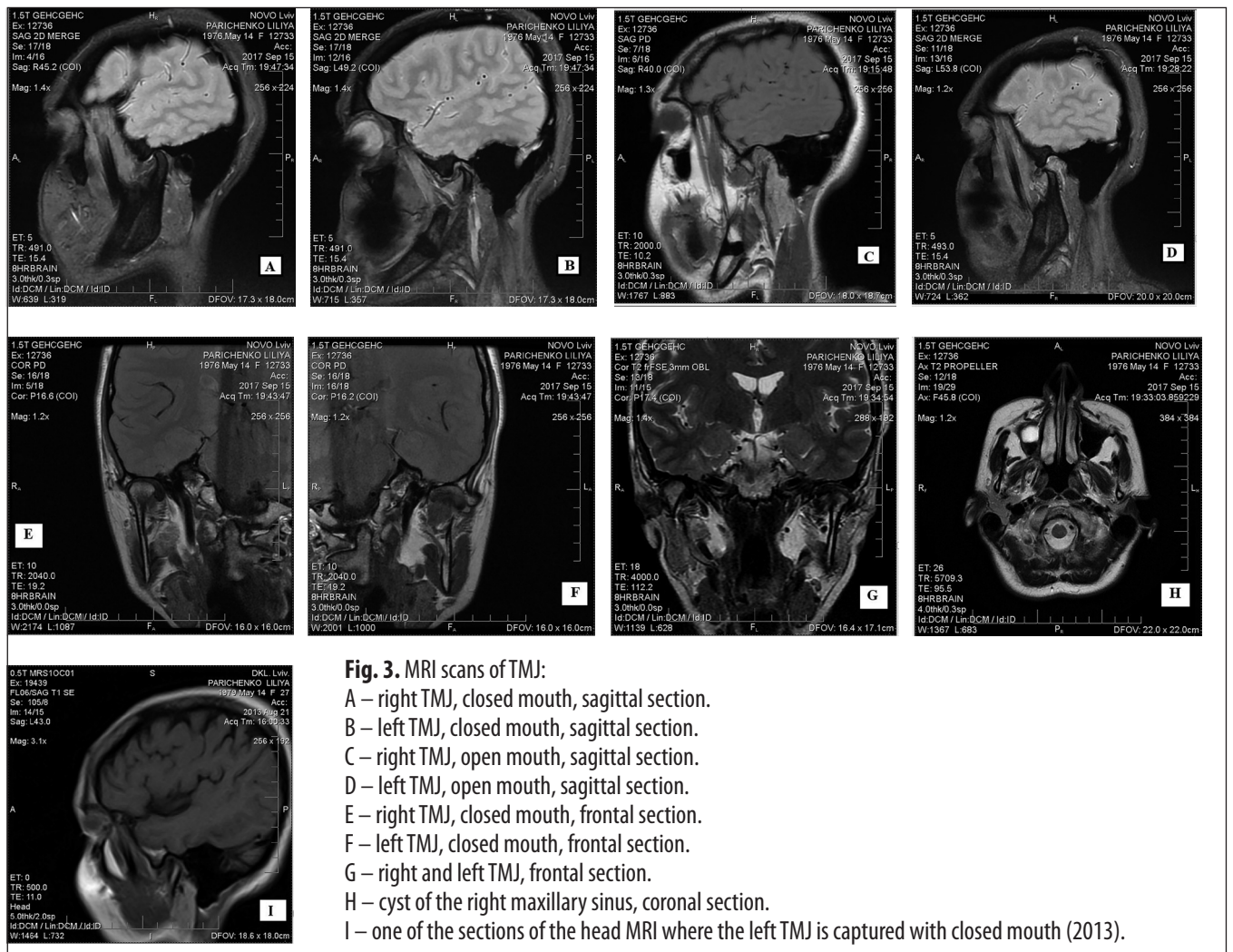**Fig. 2.** Diagnostic orthopantomogram (2011)



**Fig. 3.** MRI scans of TMJ:
A – right TMJ, closed mouth, sagittal section.
B – left TMJ, closed mouth, sagittal section.
C – right TMJ, open mouth, sagittal section.
D – left TMJ, open mouth, sagittal section.
E – right TMJ, closed mouth, frontal section.
F – left TMJ, closed mouth, frontal section.
G – right and left TMJ, frontal section.
H – cyst of the right maxillary sinus, coronal section.
I – one of the sections of the head MRI where the left TMJ is captured with closed mouth (2013).

MRI scan of the brain for 2013 revealed that the deformity of the condyle took place back then (Fig. 3-G). It should be noted that orthopantomograms for 2017 and 2011 did not reveal any deformities of the condyle, which confirms the relevance of a thorough clinical and follow-up radiological surveys.

The patient was referred for additional consultations to rheumatologist, ENT doctor due to maxillary sinus cyst, and dental therapist to rule out or confirm the odontogenic origin of maxillary sinus cyst. The patient was prescribed a number of follow-up examinations, including maxillofacial CT. Some CT sections are shown in Figure 4.

After a course of drug therapy prescribed by rheumatologist and endocrinologist, mandibular stabilization bite splint to be used at night and 1 hour during the day was made for the patient. Clinical picture after 2 months of using the bite splint and its regular corrections: opening of mouth for 50 mm, normal movement trajectory, no discomfort at opening of mouth, absence of response to the provocation compression test of TMJ. A control USG
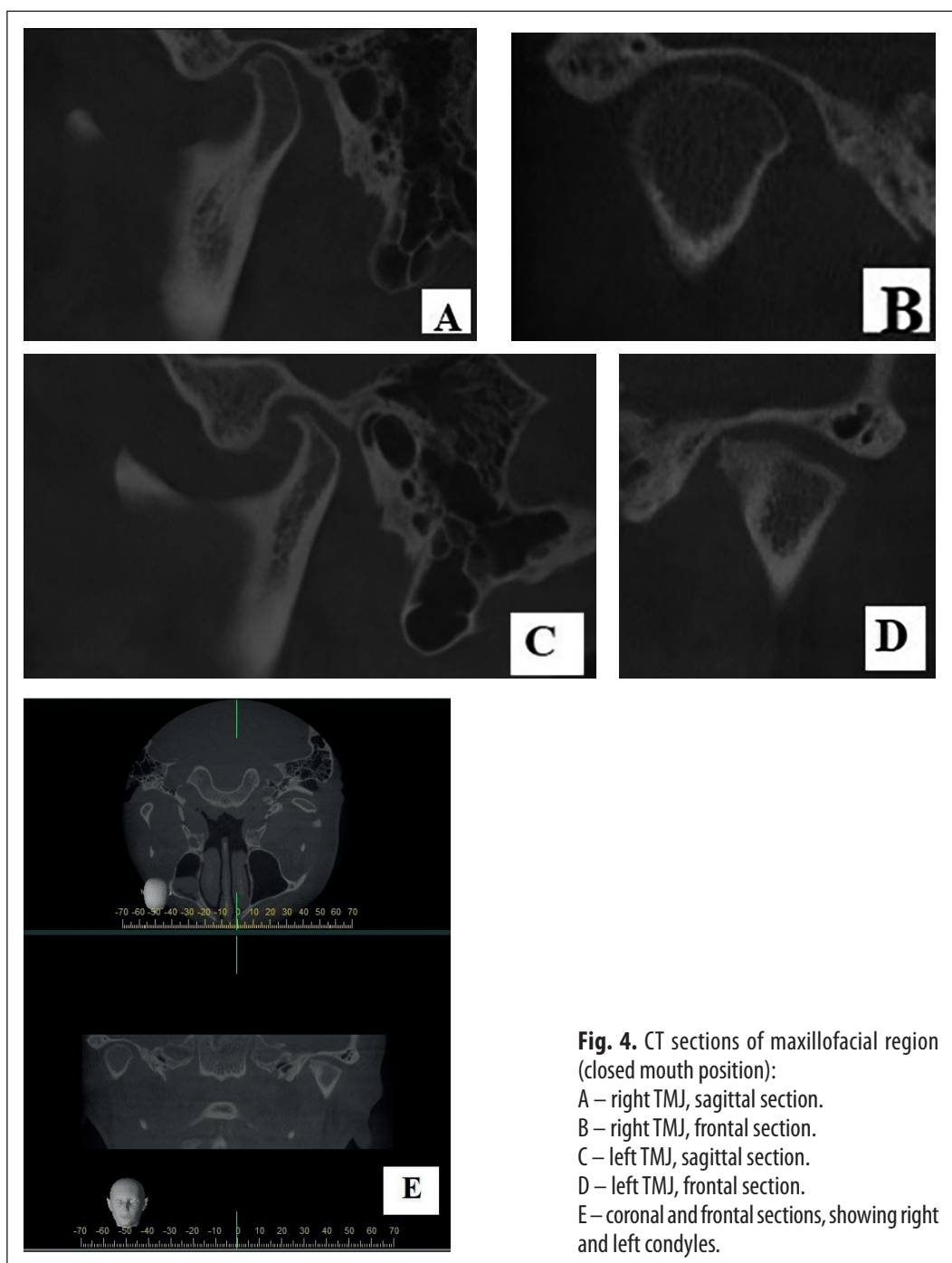
**Fig. 4.** CT sections of maxillofacial region
(closed mouth position):
A – right TMJ, sagittal section.
B – right TMJ, frontal section.
C – left TMJ, sagittal section.
D – left TMJ, frontal section.
E – coronal and frontal sections, showing right
and left condyles.

examination reveals normal discs positioning, the same initial degenerative changes of the head of the left TMJ, amplitude in both joints is 16 mm.

## ULTRASONOGRAPHY

Visualization of TMJ and disc using USG was first reported by Nabeih and Speculand [19]. Stefanoff et al. reported successful results of evaluation of the TMJ disc in asymptomatic volunteers using USG [20]. Study of *Bas et al. has shown* that USG *provided a sensitivity of 69%* in the *detection of internal* derangements, *while high resolution* ultrasonography (*HR-US*) *showed a sensitivity between 65-95%* in the *determination of TMJ disorders* [21-24].

Other researchers think that examination of temporomandibular joint with the help of high-frequency ultrasound is a diagnostic method of unconfirmed effectiveness. The majority of studies focused on assessing pathological conditions rather than the condition of healthy joints to determine the correct USG image [25]. Dynamic ultrasonography turned out to be a reliable diagnostic tool for detection of normal disc position. The results of this study are of additional interest and should encourage research as to its potential uses and diagnostic capabilities [26]. A large number of studies in the literature deal with the use of USG for determination of anterior disc displacement. However, researchers draw attention to the failure of ultrasonography

to detect lateral and posterior displacements [12]. Nevertheless, there is a need for an inexpensive, non-invasive and simple diagnostic technique for temporomandibular joint imaging. High-frequency ultrasonography seems to be promising thanks to technological advances that provide more powerful transducers [25].

The principle of ultrasonography is based on the fact that the ultrasonic waves emitted by the device (transducer) pass through TMJ and are partially reflected in transit through various anatomical structures. Then the reflected sound waves are read by the same emitting device and transferred to the image [27,28]. The possibility of conducting dynamic survey, which enables to evaluate smooth function of TMJ, movement amplitude of condyles, the synchronism of motion of the head and disc is its feature and the greatest advantage. Ease of execution, accessibility, low invasiveness, unchanged normal posture of a patient, absence of radiation exposure and higher density of units if compared to MRI contributes to the increasing use of USG in the diagnosis of TMD [28,29]. Scope of diagnostic information obtained with the use of ultrasound combined with orthopantomography or computed tomography is meaningful enough for the diagnosis at the initial stage [8,30]. Appropriate qualifications of operators allow obtaining satisfactory result and minimizing discrepancies in the analysis of diagnostic data.

The TMJ area comprises various structures that reflect sound waves in different ways. The bone tissue represented by the head of the articular process and the articular tubercle is usually hypoechoic (low reflection of sound waves). Its images on ultrasonography are black, but the edge of the bone is hyperechoic (strong reflection of sound waves) and appears white in ultrasonographic images. The connective tissue represented by the articular capsule and the retrodiscal tissue (bilaminar zone), the muscular tissue represented by the lateral pterygoid and masticatory muscles, are isoechoic (intermediate reflection of sound waves) and appear unevenly gray on ultrasonography images. However, the surface of the articular capsule and the surface of the muscles strongly reflect sound waves that generate a hyperechoic (white) line. Empty space and water (upper and lower chambers) of the joints are hypoechoic and appear black on ultrasonographic images, but these anatomical cavities are virtual, because the opposite surfaces are in contact and are usually not detected during the examination if there is no effusion. The disc, like all major ligaments, consists of dense fibrous tissue, but its appearance on ultrasonographic images is contradictory [27].

Ultrasonography is performed in two positions: in the supine and in the sitting positions, in a state of habitual occlusion. During the examination, the patient's head is turned in the direction opposite to the site under examination. To evaluate the condition of the TMJ structures, three main approaches are used: a horizontal scan (the transducer is placed under zygomatic arch at an angle of 38-45 ° to the horizontal plane) and two frontal scans: posteriad and anteriad to the head of mandible, respectively, the transducer is placed in frontal plane under zygomatic arch at an angle of 45° anteriad [31].

At the first stage of the study, the examination, performed in horizontal scan, evaluates the upper lateral region of the head of mandible; the lateral fragment of the articular capsule; the presence of joint fluid; capsule-condyle distance; the lateral fragment of the articular disc and its position; the acoustic shadow from the bone tissue of the articular tubercle; the lateral pterygoid muscle (upper and lower heads); the masticatory muscle (superficial and deep regions); periarticular soft tissues. At the same time, scans perform a functional test (opening and closing of mouth) in order to determine movement amplitude of the head of mandible [31].

The second stage is the examination in the frontal scan in two positions: posteriorly from the head of mandible and anteriorly from the head of mandible. The transducer is placed in the frontal plane under zygomatic arch at an angle of approximately 45°, posteriad and anteriad to the head of mandible respectively. In this case the posterior lateral region of the head of mandible, the neck of the articular process of mandible, the posterior margin of the branch of mandible, the posterolateral fragment of the articular capsule, the bilaminar zone, the lateral part of the posterior (anterior) portion of the articular disc are evaluated. Disc visualization cannot be obtained when the head of mandible and disc are deeply located [31].

In case of degenerative changes of the joint, ultrasonography detects bone growth (osteophytes) on the surface of the head of mandible, thickness loss of subchondral-cartilaginous complex, subchondral cysts, its roughness and fragmentation. The reduction in capsule-condyle distance is assessed in both horizontal and frontal scans. In the presence of effusion in the joint, an increase in its dimensions is established, which is a confirmation that degenerative changes of TMJ may be accompanied by inflammatory process. Also, a decrease in the movement amplitude of the condyle and asynchrony of motion of the head and disc at maximal opening of mouth are found [31].

Informative value of different study methods, depending on hard or soft tissues of the joint involvement [2] is presented in Table I.

Magnetic resonance imaging is based on the use of electromagnetic radiofrequency non-ionizing radiation. In order to obtain an image, the patient is placed inside a strong magnetic field, which causes setting of the hydrogen atoms nuclei protons in accordance with the polarity of the field. With the use of radio signal, energy magnitude released is utilized for computer reconstruction of magnetic resonance imaging [31, 4].

During magnetic resonance imaging of TMJ, T1 and T2 – weighted images with closed and open mouth are made. T1-weighted images are made in order to visualize bone tissue and articular disc. T2-weighted images are made to detect inflammatory changes and fluid in TMJ. With the help of the "rapid scan" function, one can examine TMJ during opening and closing of mouth, i.e. in motion. MRI is also informative for changes in ligaments, articular discs and masticatory muscles [32-34].

MRI allows high performance determination of such pathologies as anterior displacement of the articular disc

with or without reduction, disc displacement in the medial and lateral directions in the coronal (frontal) plane. T2 image better demonstrates possible myxoid (mucosal) degeneration of the articular disc, but this sequence requires more time for MRI survey. The posterior adhesion of the articular disc (bilaminar zone) of TMJ is a highly plastic tissue involved in the redistribution of fluid (blood, tissue fluid, synovial fluid), able to change its volume in a wide range. With the use of MRI, one can assess its structure and volume depending on the position of the condyle. MRI is used to study the function of the masticatory muscles at rest and in dynamics. Edema and effusion in the lateral pterygoid muscle may be detected in 80% of patients with TMD on T2-weighted images. Thus, MRI leaves behind all other methods of study in many respects [31].

This method provides the best resolution in visualization of TMJ tissues, being non-invasive, not based on the use of ionizing radiation (complete safety for the patient and the staff), enables to obtain a multiplanar image without moving the patient, natural contrast of moving blood and absence of artifacts of bone elements [31].

Disadvantages of this method include long examination time, restricted use in patients with pacemakers, implants containing ferromagnetic alloys, in pregnant women, as well as in patients with claustrophobia, and the high cost of diagnosing [31]. The MRI method does not allow assessing both the TMJ function and its structure [18,25]. Examination with opening and closing of mouth is not informative due to the complexity of technological process and low frequency [8]. Quality of the results obtained is directly dependent on the qualifications of operators, their skills and experience of work with the unit.

## CONCLUSIONS

MRI remains the recognized "gold standard" for the diagnosis of temporomandibular disorders. However, despite the lack of sufficient number of scientific research, ultrasonography fully satisfies the needs of primary diagnosing. Its combination with one of radiological diagnostic methods gives a comprehensive picture of the state of the structures of temporomandibular joints. The particular advantage of USG over all other methods is the procedure in dynamics and in real time, giving unique data on joint function. Upon comparison of the results of USG and MRI described in the clinical case report, we can conclude that USG is sufficiently sensitive and specific. Its basic utilization during the initial examination gives a doctor an orientation for further diagnosis and follow-up consultations. Attention is drawn to the fact of high quality assessment of the state of subchondral-cartilage complex and an early-stage degenerative changes of the condyle with the help of USG, which can be used to clarify the diagnosis in patients with restricted use of MRI examination. Absence of harm to a patient allows the use of this method often to monitor management, while the complexity and restricted application of MRI do not always justify results; so it does not always make sense. The study of ultrasonography as a diagnostic tool in the detection of

TMD remains open and requires further research. However, MRI should be used, if possible, for acquisition of knowledge of TMJ structures visualization, gaining in experience by radiologists and an increased application of MRI in dentistry.

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CASE STUDY

# DIFFERENTIAL DIAGNOSIS OF PAROXYSMAL STATES: LITERATURE REVIEW AND ANALYSIS OF A CLINICAL CASE ON THE EXAMPLE OF CLOCCS-SYNDROME IN A YOUNG MAN

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**ABSTRACT**
Diagnosis of paroxysmal conditions in neurology is one of the most difficult problems. Particular difficulties are caused by differential diagnosis of epileptic and non-epileptic paroxysmal states. There are no absolutely pathognomonic signs of epileptic and non-epileptic seizures. False positive diagnosis of epilepsy occurs in 2-71% of cases.

Diagnosis of paroxysmal conditions requires an integrated approach to the problem and includes not only a clinical examination, but also a thorough history taking, neurophysiological, neuroimaging, laboratory research methods, involves the involvement of other specialists.

The article presents a clinical case of 27-year-old young man who was initially misdiagnosed. Using the methods of functional and laboratory diagnostics, the patient was diagnosed correctly. Instead of idiopathic epilepsy, he was diagnosed with cytotoxic lesions of the corpus callosum (CLOCCs-syndrome associated with an infectious process) with motor paroxysms of non-epileptic genesis.

Thus, using the example of this clinical case, it has been shown that the differential diagnosis of epileptic and non-epileptic paroxysmal states presents significant difficulties for a practicing neurologist.

**KEY WORDS:** epilepsy, non-epileptic states, diagnostic, Epstein-Barr virus, CLOCCs-syndrome

## INTRODUCTION

The paroxysmal conditions are one of the most common pathological disorders in the daily practice of a neurologist. The *paroxysm* is usually understood as a sudden deterioration in health, which is manifested or accompanied by episodes of illness, or a sharp, short-term (more often repeated) exacerbation of a chronic painful condition. The paroxysmal conditions, as a rule, are not independent nosological forms, but represent a manifestation of a particular pathology of the central nervous system, but it is the presence of paroxysmal symptoms that often causes seeking medical help. These conditions are not only a medical problem, but also a social problem [1].

The urgency of the problem of paroxysmal pathology is due to the fact that some paroxysms threaten the lives of patients, and most of the paroxysmal conditions are disabling.

According to the classification according to V. A. Karlov, all paroxysms are divided into:
1) epileptic;
2) non-epileptic (syncopal states, fainting, collapse, stress attacks with compression of the vertebral artery, facial paroxysms, hypo- and hyperkaliemic myoplegia, myasthenic crisis, as well as vegetative disorders, muscle

dystonia and other paroxysmal motor diseases, restless legs syndrome and periodic movements in sleep, various paroxysmal neuralgia) [2].

Non-epileptic states can be defined as sudden, destructive, changing behavior, sensitivity, thinking, sensations that are usually limited in time and similar to or mistaken for epileptic seizures [3].

These paroxysmal disorders are extremely heterogeneous in nature. A significant part of them is associated with neuroses and psychogenic disorders. However, somatogenic disorders (migraine, panic attacks, vestibular paroxysms, hyperparathyroidism, etc.) occupy an equally significant place in the genesis of these conditions [4].

A seizure is the transient manifestation of abnormal excessive or synchronous electrical brain activity that causes convulsions, loss of consciousness, and or lapses of consciousness. The underlying cause of seizures is a state of neuronal hyperexcitability that may be temporary (e.g., due to electrolyte imbalances) or more permanent in nature (e.g., due to inherited or acquired neural abnormalities). Seizures can be triggered by a variety of circumstances depending on age, environmental factors, and underlying conditions. Acute symptomatic seizures (provoked seizures) have identifiable precipitating factors (e.g., stroke,

**Table I.** Probability of error in patients with suspected epilepsy according to population and cohort studies

| Author | Presumably the diagnosis of epilepsy | Misdiagnosis | CVD syncope |
|---|---|---|---|
| Scheepers B.<br>(Seizure 1998) | 261 | 49 | 15 |
| King M.A.<br>(Lancet 1998) | 496 | 178 | 60 |
| Smith D.<br>(QJM 1999) | 184 | 46 | 13 |
| Grubb B.P.<br>(Ann Intern Med 1991) | 15 | 10 | 10 |
| Linzer M.<br>(Am J Med 1994) | 12 | 12 | 5/7 |
| Zaidi A. (JACC 2000) | 74 | 31 | 29 |

traumatic brain injury, alcohol withdrawal), whereas unprovoked seizures occur in the absence of identifiable causes. Reflex seizures are states that occur consistently in response to a particular trigger [5].

The problem of differential diagnosis of paroxysmal states is one of the most difficult tasks in neurology. It is due to a number of subjective and objective reasons: an insufficiently complete and accurate description of the clinical features of the seizure by patients and their relatives, often low information content of additional laboratory and instrumental research methods, the transient nature of disorders and often the absence of objective symptoms in the interparoxysmal period, insufficient awareness of doctors and medical staff about the clinical features of a number of paroxysmal conditions, etc. Among neurological paroxysmal conditions, the most difficult, requiring highly qualified clinicians is the differential diagnosis of epileptic and non-epileptic states. Sudden disturbances of consciousness, paroxysmal motor disorders, paroxysmal changes in behavior, crisis vegetative states are often mistaken for epilepsy [6].

Published retrospective and prospective studies suggest that 1 out of 4 patients with "epilepsy" was mistakenly diagnosed based on the analysis of clinical manifestations and the results of tilttest.

According to leading epileptologists, up to 20-30 % of patients diagnosed with epilepsy and receiving antiepileptic treatment suffer from non-epileptic states. Up to 45 % of patients diagnosed with refractory epilepsy have non-epileptic states (Table I).

Y. Xu et al (2016) in their review cite data indicating a significant number of false diagnostics of epilepsy even in specialized highly qualified medical institutions (Fig. 1) [7].

The diagnosis of paroxysmal states in neurology is one of the most difficult problems. The differential diagnosis of epileptic and non-epileptic paroxysmal states causes particular difficulties, due to the lack of absolutely reliable clinical markers of the disease in the interparoxysmal period, not always sufficient information content of instrumental research methods and insufficient awareness of doctors [8].

The problem of differential diagnosis of paroxysmal conditions is difficult, since it belongs to the category of multidisciplinary. The he main problems of differential diagnosis of paroxysmal states lie in the field of neurology, cardiology and psychiatry in patients [8].

The article presents a clinical case of an erroneous interpretation of the diagnosis in a 27-years-old young man, taking into account complaints, anamnesis of the disease and life, neurological status, laboratory results, features of the EEG, MRI.

## ETHICAL ASPECTS
The work complies with the ethical standards of the Declaration of Helsinki by the World Medical Association. A written informed consent was obtained authorizing the publication of the medical history and the results of the examination.

## CASE REPORT
Patient P., 27 years old (06.08.1993), was admitted to the military hospital on 08.04.2021 with complaints of periodic, with a frequency of 1-2 times a month, attacks of tonic tension of the muscles of the right extremities, which last several seconds, are provoked by active movements.

It is known from the anamnesis of the disease that periodic attacks of tension in the right extremities have been bothering since the age of 12, the patient has not sought medical help until now.

The patient notes the deterioration of the condition in the form of increased frequency of seizures and an increase in their duration for the last 2 years.

He consulted a unit doctor in March 2021. An MRI scan of the brain was performed, it was not found pathology. The patient was referred to the neurological department of military unit for further examination and treatment.

At the time of the examination, the patient's general condition is satisfactory. A man for a regular physique. The skin is clean, normal color. The visible mucous membranes are normal color. The thyroid gland is not enlarged, it is painless. Peripheral lymph nodes are not enlarged. Above the lungs, a pulmonary sound is heard,
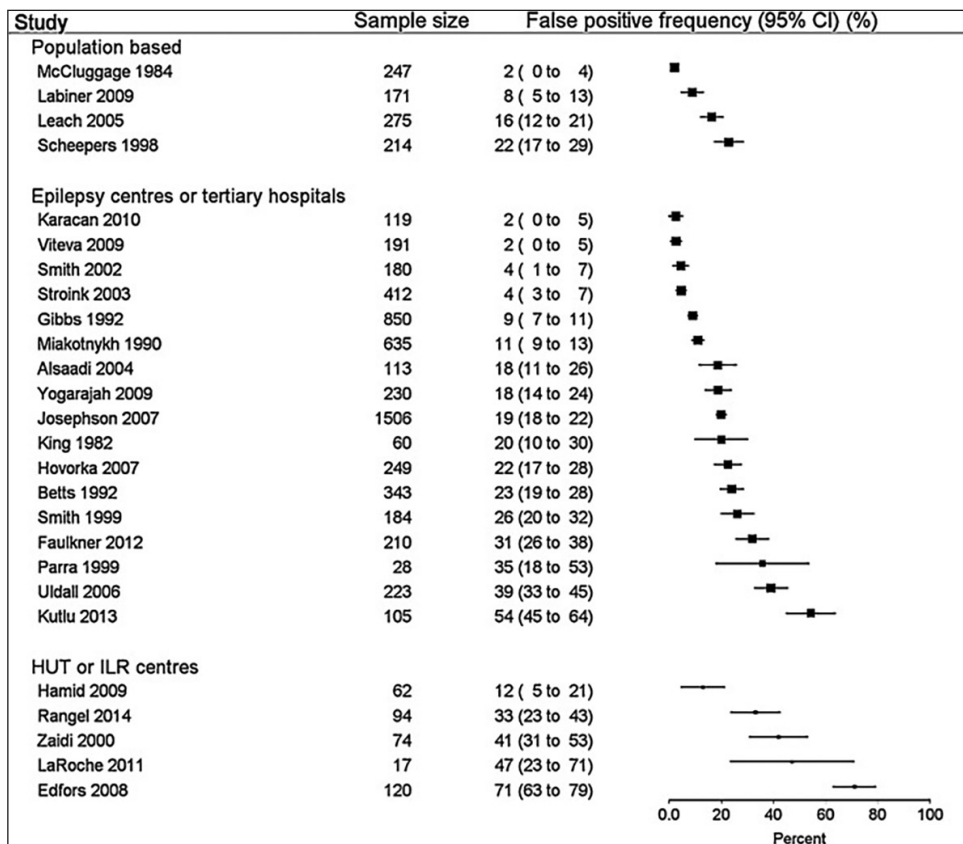
| Study | Sample size | False positive frequency (95% CI) (%) | |
|---|---|---|---|
| **Population based** | | | |
| McCluggage 1984 | 247 | 2 ( 0 to 4) | |
| Labiner 2009 | 171 | 8 ( 5 to 13) | |
| Leach 2005 | 275 | 16 (12 to 21) | |
| Scheepers 1998 | 214 | 22 (17 to 29) | |
| **Epilepsy centres or tertiary hospitals** | | | |
| Karacan 2010 | 119 | 2 ( 0 to 5) | |
| Viteva 2009 | 191 | 2 ( 0 to 5) | |
| Smith 2002 | 180 | 4 ( 1 to 7) | |
| Stroink 2003 | 412 | 4 ( 3 to 7) | |
| Gibbs 1992 | 850 | 9 ( 7 to 11) | |
| Miakotnykh 1990 | 635 | 11 ( 9 to 13) | |
| Alsaadi 2004 | 113 | 18 (11 to 26) | |
| Yogarajah 2009 | 230 | 18 (14 to 24) | |
| Josephson 2007 | 1506 | 19 (18 to 22) | |
| King 1982 | 60 | 20 (10 to 30) | |
| Hovorka 2007 | 249 | 22 (17 to 28) | |
| Betts 1992 | 343 | 23 (19 to 28) | |
| Smith 1999 | 184 | 26 (20 to 32) | |
| Faulkner 2012 | 210 | 31 (26 to 38) | |
| Parra 1999 | 28 | 35 (18 to 53) | |
| Uldall 2006 | 223 | 39 (33 to 45) | |
| Kutlu 2013 | 105 | 54 (45 to 64) | |
| **HUT or ILR centres** | | | |
| Hamid 2009 | 62 | 12 ( 5 to 21) | |
| Rangel 2014 | 94 | 33 (23 to 43) | |
| Zaidi 2000 | 74 | 41 (31 to 53) | |
| LaRoche 2011 | 17 | 47 (23 to 71) | |
| Edfors 2008 | 120 | 71 (63 to 79) | |

0.0  20  40  60  80  100
Percent

**Fig. 1.** Observational studies of the frequency of false positive diagnosis of epilepsy
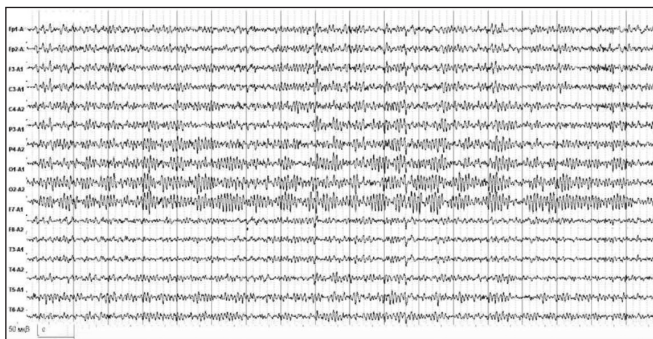


**Fig. 2.** The EEG fragment of patient P., 1993



**Fig. 3.** The EEG-video monitoring fragment of patient P., 1993. Rec=23-05-2021 16-58 {All record}

vesicular breathing. The pulse is rhythmic, heart rate is 66 beats/min, blood pressure is 120/80 mm Hg. The boundaries of the heart are within the normal range. The heart tones are clear, the rhythm is correct. The abdomen is soft, painless on palpation. The liver and spleen are not enlarged. Pasternatsky symptom is negative on both sides. There are not edemas. Physiological functions are controlled.

In the neurological status: the level of consciousness is clear, it is oriented correctly in time and place, intelligence corresponds to age. He is available to the productive contact in full. Meningeal signs are negative. Palpebral fissures, pupils are D=S. The light reflex is of average vivacity. The movements of the eyeballs are possible in full. Convergence is weakened. Nystagmus, diplopia are absent. The nasolabial folds are symmetrical. The pharyngeal reflex is triggered, swallowing is not disturbed. The tongue is moist, is not

overlaid, along the middle line. Speech is not broken. Phonation is normal. There are not bulbar violations. Muscle tone is preserved. Muscle strength is sufficient. Tendon and periosteal reflexes from the hands D=S, knee D=S, Achilles D=S, of average vivacity. There are not pathological feet signs. Sensitive disorders are not detected. The finger and knee-heel tests are performed satisfactorily. He is persistent in the Romberg pose. The coordination tests are performed satisfactorily. The vegetative reactions are revived.

During the entire period of stay in the military hospital, the following studies were conducted:

There were not significant deviations from the reference values in general clinical blood and urine tests.
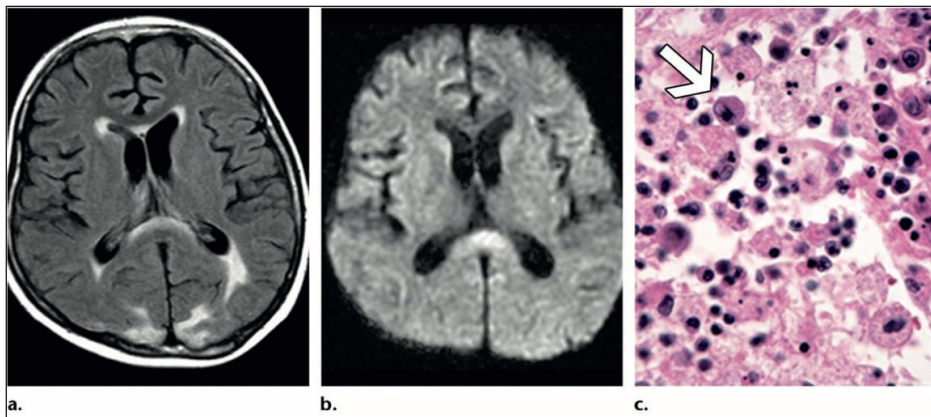
**Fig. 4.** Infection-associated CLOCCs-syndrome in a 27-year-old man, in whom EBV infection was diagnosed. (a) Axial FLAIR MR image shows a hyperintense oblong splenial lesion and mild involvement of the anterior corpus callosum. (b) Axial diffusion-weighted MR image shows reduced diffusion in the callosal lesions. (c) High-power photomicrograph shows infiltration with hemophagocytic histiocytes (arrow) and atypical lymphocytes, findings consistent with EBV-associated hemophagocytic lymphohistiocytosis. (H-E stain; original magnification, ×400).
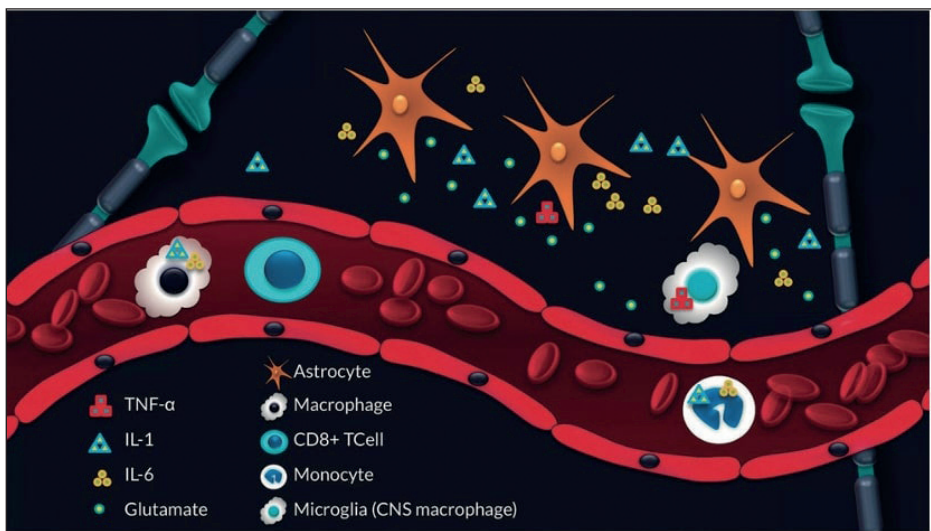


**Fig. 5.** Drawing shows the cells and cytokines that are important in the development of CLOCCs. Cell-cytokine interactions leads to massively elevated extracellular glutamate levels
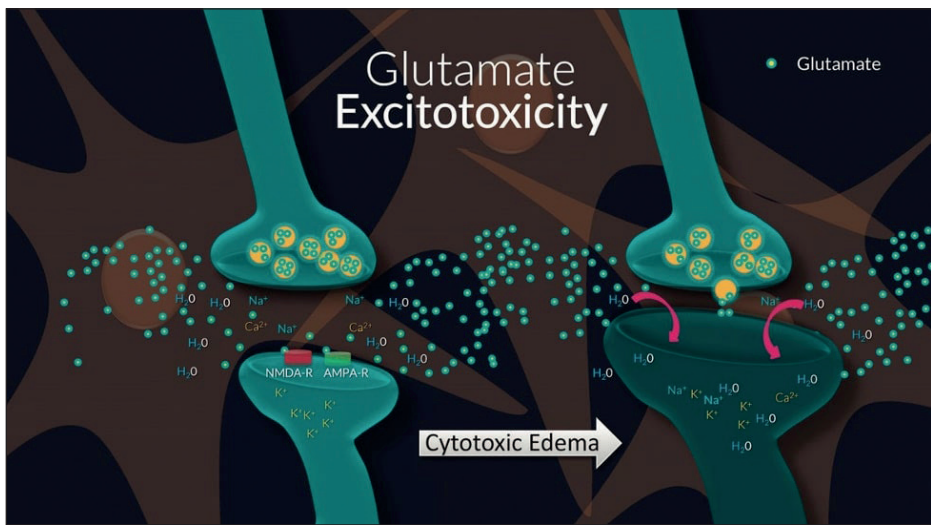


**Fig. 6.** Drawing shows glutamate excitotoxicity. The extracellular glutamate binds with N-methyl-D-as- partate receptors (NMDA-R) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA- R), allowing sodium ions (Na+) and calcium ions (Ca+) to enter cells, potassium ions (K+) to leave cells, and water (H2O) to enter (pink arrows) and become trapped within neurons. This process leads to cytotoxic edema. Because the intracellular water cannot freely move, cytotoxic edema is manifest as reduced diffusion at MR imaging

Biochemical blood assay (09.04.2021): ACT 18,7 Od/L; ALT 16,1 Od/L; blood urea 2,9 mmol/L; creatinine 91,3 mcmol/L; blood glucose teste 5,3 mcmol/L; LPH 55,7 Od/L.

SARS-CoV-2 antibody assay to the nucleocapsid antigen (09.04.2021): IgM and IgG were not detected.

Thyroid hormones (13.04.2021): T4 – 18,8 mmol/L; TSH – 1,09 мсMO/mL; TPO – 11,8 Od/mL.

Rapid Plasma Reagin test (09.04.2021): negatively.

ECG (08.04.2021): sinus rhythm. Early Ventricular Repolarization Syndrome.

Fluorography of chest organs (09.04.2021): without pathological findings.

EEG (15.04.2021): pathological EEG complexes have not been registered.

ECHO (13.04.2021): without pathological findings.

Diagnostic ultrasound of thyroid (13.04.2021): without pathological findings.

Head MRI (27.03.2021): on the hands.

Against the background of a complete collection of complaints, anamnesis of the disease and life, as well as the

results of laboratory and instrumental research methods, this patient was diagnosed with «Idiopathic epilepsy with simple partial tonic seizures. S-shaped scoliosis of the thoracic spine of the first degree» [8], [17].

The patient was prescribed treatment: compliance with the regime, diet, taking carbamazepine 0.2 2 tablets 2 times a day.

During the treatment in the military hospital, the patient did not notice a significant improvement. Isolated seizures of tonic tension of the muscles of the right extremities were recorded. The patient's condition is satisfactory.

The patient is recommended to avoid physical and emotional overloads, observe the work and rest regime, follow up with a neurologist, take levicitam 250 mg 2 times a day for 2 weeks, then 500 mg 2 times a day for a long time. The patient was discharged from the military hospital on 23.04.2021.

Subsequently, patient P. was consulted by a neurologist on 06.05.2021. The patient complained of periodic seizures in the right extremities in the form of muscle contractions, which are provoked by emotional and physical exertion. The patient has paroxysmal states of unclear genesis, which need to be clarified. It has not the features in the neurological status of a patient. It is recommended to do a diagnostic head MRI with 3 T – the presence of cortical dysplasia, the presence of focal pathology; and also to do EEG and EEG video monitoring (24 hours) with the cancellation of levicetam 4 days before the start of the examination. The next inspection after the additional examination.

The patient underwent an EEG (06.05.2021): a medium-voltage EEG torrent with dominant δ-activity. The main violations of bioelectric activity in the alpha-rhythm range, the correctness of its zonal distribution, amplitude values and fusiformity are preserved, the alpha-rhythm is expressed by flashes of up to several seconds, separated from each other by sections of low-amplitude polymorphic slow activity, the intervals between alpha-rhythm flashes are filled with a flat EEG, generalized flashes of slow-wave activity, mainly in the central leads, against the background of normal electrical activity. It were not detected the specific EEG phenomena (Fig. 2) [14], [15].

The patient performed EEG video monitoring (24 hours) on 23.05.2021. The following conclusion was obtained: diffuse disturbances in the bioelectrical activity of the brain and local changes were not registered. Single epileptiform discharges were recorded in the inferior forehead-temporal region. Revealed dysfunction of the median structures of the brain. The seizures recorded during the survey are most likely non-epileptic in nature (Fig. 3) [14], [15].

*Head MRI 3 T* (epileptic protocol) (09.05.2021): MR-sings of cytotoxic lesions of the corpus callosum (CLOCCs-syndrome). MR-signs of mesial temporal sclerosis and cortical dysplasia of the brain were not detected (Fig. 4).

*The neurologist consultation* (31.05.2021): The complaints are the same. It has not the features in the neurological status of a patient. Taking into account the data of instrumental research methods, the patient was diagnosed with "Cytotoxic lesion of the corpus callosum (CLOCCs-syn-

drome) with motor paroxysms of non-epileptic genesis". It were recommended: consultation and examination by an infectious diseases specialist-virologist. Levetiracetam should be gradually discontinued.

After consultation with a neurologist, the patient turned to the laboratory and donated blood to the infection panel, in particular for the presence of antibodies to the Epstein-Barr virus.

Assay for the presence of IgG antibodies to the Epstein-Barr virus capsid antigen VCA (EBV VCA IgG) (04.06.2021): 15,39 Od.

Assay for the presence of IgM antibodies to the Epstein-Barr virus capsid antigen VCA (EBV VCA IgM) (04.06.2021): 0,31 Od.

Assay for the presence of IgG antibodies to the Epstein-Barr virus nucleonic antigen (EBV EBNA-1 IgG) (04.06.2021): 12,82 Od.

Thus, the constant presence of VCA IgG in high titers indicates a chronic phase of infection caused by the Epstein-Barr virus. The reason for the positive result may be the presence of active immunity due to a previously transmitted infection, along with the detection of antibodies to the nuclear antigen (EBNA) and the absence of IgM to the capsid antigen (VCA) of the Epstein-Barr virus.

After another visit to the neurologist, the patient was diagnosed with the following: "The consequences of neuroinfection in the form of cytotoxic lesions of the corpus callosum (CLOCCs-syndrome) with motor paroxysms of non-epileptic genesis. Viral infection caused by the Epstein-Barr virus is in remission".

An example of differential diagnosis of paroxysmal states using instrumental methods was presented to Your attention. This clinical case demonstrated how, thanks to the methods of functional diagnostics, the patient's diagnosis was changed from idiopathic epilepsy to CLOCCs syndrome of non-epileptic genesis associated with infection.

Cytotoxic lesions of the corpus callosum (CLOCCs) is a concept that combines a heterogeneous group of pathological conditions that cause changes in the signaling characteristics of the corpus callosum, in particular, the roller [9].

Cytotoxic lesions of the corpus callosum (CLOCCs) represent a group of conditions that cause MRI signal intensity changes in the corpus callosum. Etiology of this phenomenon is very heterogenous. CLOCCs are associated with a spectrum of metabolic disorders, drug therapy, infections, epileptic seizures and many other causes.

Complex interdependent mechanisms regulate cytokine levels and, ultimately, glutamate levels in the brain. With trauma, infection, and inflammation, macrophages become active and release the inflammatory cytokines interleukin 1 (IL-1) and IL-6, beginning the cascade that leads to cytokinopathy. Monocytes then activate and also release IL-1 and IL-6. T-cells are subsequently recruited and affect the endothelial cells, making the endothelial cells leaky (breaking down the blood-brain barrier) and stimulating them to produce tumor necrosis factor-α (TNF-α). Astrocytes, in turn, are stimulated by IL-1 to release glutamate and block reuptake of glutamate, thus increasing extracellular gluta-

mate. Microglia, which are the macrophages of the central nervous system (CNS), subsequently become activated and produce more cytokines and may initiate demyelination. Many of these cell-cytokine relationships include feedback loops that are exponentially amplified. The result of this cytokinopathy is massively increased amounts of glutamate in the extracellular space at levels 100 times the normal level or more (Fig. 5) [10].

The CLOCCs demonstrate increased signal intensity on fluid-attenuated inversion-recovery (FLAIR) magnetic resonance (MR) images and show decreased signal intensity on T1-weighted MR images [11]. Diffusion is reduced (mean ADC value, $0.31 \times 10^{-3}$ mm²/sec; range, $0.13 \times 10^{-3}$ mm²/sec to $0.48 \times 10^{-3}$ mm²/sec). CLOCCs lack enhancement on contrast material–enhanced images, tend to be midline, and are relatively symmetric. The involvement of the corpus callosum typically shows one of three patterns: (a) a small round or oval lesion located in the center of the splenium, (b) a lesion centered in the splenium but extending through the callosal fibers laterally into the adjacent white matter, or (c) a lesion centered posteriorly but extending into the anterior portion of the corpus callosum (Fig. 6).

CLOCCs are secondary lesions associated with drug therapy, malignancies, infections, subarachnoid hemorrhage (SAH), metabolic disorders, trauma, and other entities. CLOCCs demonstrate reduced diffusion from cytotoxic edema. They are usually ovoid and located in the splenium but may be more extensive, with involvement of the body of the corpus callosum and the genu. CLOCCs are frequently but not invariably reversible. When they are present, their underlying cause should be sought and addressed [12], [16].

## VARIANTS OF CLOCCS BY ETIOLOGY

*I. CLOCCs associated with drugs/toxins;*
– antidepressants (amitriptyline);
– antipsychotics (clozapine);
– chemotherapeutic drugs (cyclosporine, fluorouracil);
– corticosteroids;
– pesticides (methyl bromide);

*II. CLOCCs associated with the neoplastic process;*
CLOCCs can be a consequence of a malignant process within the central nervous system. There is evidence of the association of CLOCCs with malignant tumors of other organs, which is most likely associated with chemotherapy. The probable mechanism of CLOCCs occurrence in this scenario is the infiltration of tumor cells with the subsequent release of cytokines into the CSF.

*III. CLOCCs associated with the infectious process;*
There are reports of cases of CLOCCs in brain abscesses, encephalitis and meningitis. The main mechanism is an increase in the level of pro-inflammatory cytokines, an increase in the permeability of the BBB and further events

that contribute to the development of excitotoxicity.
– viruses (influenza, measles, herpes, mumps, adenovirus, chickenpox, rotavirus);
– bacteria (salmonella, legionnaires ' disease);
– mycobacteria (tuberculous meningitis);

*IV. CLOCCs associated with metabolic disorders;*
– electrolyte disorders (hyperammonemia, hyper- and hyponatremia);
Ammonia is one of the main participants in the pathogenesis of hepatic encephalopathy. The acute toxic effect of ammonia is a massive release of cytokines and the further development of excitotoxicity, mainly mediated by NMDA receptors.
– hemolytic-uremic syndrome;
– hepatic encephalopathy;
– hypoglycemia;
– Markiafava-Bignami disease;
– osmotic demyelinating syndrome;
– Wernicke's encephalopathy;
– Wilson's disease;

*V. CLOCCs associated with subarachnoid hemorrhages;*
In patients with subarachnoid hemorrhages (SAH), increased levels of interleukins 1-beta, 6 and TNF-alpha are found in the CSF, which may be the cause of the appearance of CLOCCs.

*VI. CLOCCs associated with traumatic injury;*
The most common cause of CLOCCs in the corpus callosum is diffuse axonal damage. The restriction of diffusion in the focus in the corpus callosum is observed in the acute phase. This may be due to several reasons: (a) increased extracellular glutamate due to axon damage and (b) secondary release of cytokines and glutamate [16].

## CONCLUSIONS

Cytotoxic lesions of the corpus callosum are secondary lesions to a variety of causes, most notably metabolic diseases, seizures, infectious diseases, and drug action.

The pathophysiological processes leading to signaling changes in corpus callosum (CC) have not yet been fully understood and described. This is probably the result of a cascade of complex mechanisms, especially inflammatory changes and an increase in extracellular glutamate levels in the brain, manifesting as cytotoxic edema.

Clinical manifestations are very variable. Neurological impairment varies from mild to severe, but may also be absent. The most common non-specific symptoms are headaches or fever.

The main examination method is MR with a typical finding of diffusion restriction with correlate on apparent diffusion coefficient (ADC) maps.

The key to successful therapy is to identify and treat the cause. The lesions are reversible in most cases, and the prognosis is very good, but also depending on the underlying pathology.

As the availability of MRI has improved and the number of examinations has increased in recent years, the number of findings that can be termed cytotoxic CC lesions is also increasing. This trend will certainly continue in the future, which will allow a further understanding of the causes, origin, manifestations and other mechanisms of CLOCCs, which have not been described, with a larger number of patients.

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## Conflict of interest:
*The Authors declare no conflict of interest.*

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# Pallas
## Kliniken

Kliniki Pallas specjalizują się w okulistyce i medycynie estetycznej. Zatrudniamy ponad 350 pracowników w 18 lokalizacjach i jesteśmy jednym z wiodących świadczeniodawców w Szwajcarii. Poprzez innowacje stale rozwijamy naszą ofertę usług w tych dziedzinach. Czy to poprzez nowe metody leczenia, sprzęt, dodatkowe lokalizacje i współpracę, a może poprzez współpracę z Państwem w najbliższej przyszłości?

W celu uzupełnienia istniejącego zespołu w naszej grupie w lokalizacjach **Olten, Aarau i Solothurn** poszukujemy osoby z inicjatywą i niezależną osobowością na stanowisko

# specjalista oftalmologii (k/m/i) 60–100%

### Główne obowiązki

Specjalista oftalmologii w naszych placówkach w Olten, Aarau lub Solothurn zapewnia profesjonalną obsługę medycznych konsultacji zachowawczych. Osoba ta będzie kompetentnie wykorzystywać swoje umiejętności we współpracy z obecnymi lekarzami specjalistami. Do jej obowiązków będzie należało zapewnienie naszym pacjentom optymalnej opieki. Wraz ze zgranym zespołem będzie świadczyć usługi medyczne na najwyższym poziomie. Będzie korzystać z szerokiej sieci wybitnych lekarzy, ciągłych szkoleń wewnętrznych i zewnętrznych oraz nowoczesnego środowiska pracy.

### Profil kandydata

- Specjalizacja w dziedzinie oftalmologii
- Kilkuletnie doświadczenie w dziedzinie oftalmologii
- Przedsiębiorcze myślenie i działanie zorientowane na sukces i cel, z wysokim zrozumieniem jakości i obsługi
- Wysoka inteligencja emocjonalna i odporność w kontaktach z pacjentami, pracownikami oraz innymi partnerami wewnętrznymi i zewnętrznymi
- Umiejętność szybkiego rozpoznawania problemów i samodzielnego opracowywania rozwiązań
- Wybitne kompetencje doradcze w języku niemieckim, wyrażane w mowie i piśmie w sposób zrozumiały i adekwatny do adresata

### Nasza oferta

W ramach udzielania konsultacji udostępniamy nowoczesną infrastrukturę, w której można profesjonalnie leczyć pacjentów, zarówno ambulatoryjnie, jak i stacjonarnie. Zapewniamy optymalne i jak najlepsze wsparcie podczas pracy. Oprócz uregulowanych godzin pracy (brak dyżurów nocnych i niedzielnych), które pozwalają na spędzanie czasu z rodziną i czas wolny, oferujemy także możliwości szkoleń wewnętrznych i zewnętrznych. Chętnie udzielimy dodatkowego wsparcia podczas stawiania pierwszych kroków w Szwajcarii.

Wykorzystując swoje wieloletnie doświadczenie, innowacyjność, a przede wszystkim umiejętność aktywnego słuchania, mogą Państwo naszym pacjentom pozwolić odczuć wyraźną różnicę.

Szukają Państwo wszechstronnej i ciekawej pracy w rodzinnej grupie przedsiębiorstw? Prosimy o przesłanie swojej aplikacji.

Dodatkowych informacji udziela pan Melvin Fankhauser, HR Recruiter/doradca HR ds. lekarzy, tel. +41 58 335 31 84 lub e-mail: melvin.fankhauser@pallas-kliniken.ch

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