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SERUM LIPID PROFILE AND ARTERIAL STIFFNESS IN NON-DIPPERS

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Background. *A non-dipping blood pressure profile (NDP) is associated with increased arterial stiffness and other cardiovascular target organ damage. Serum lipid profiles have been shown to be important determinants of arterial stiffness.*

Objective. *The aim of the research was to assess serum lipid profiles and arterial stiffness in non-dippers.*

Methods. *This cross-sectional study was conducted involving 796 (288 males and 508 females) participants of black African origin. A twenty-four-hour ambulatory BP monitoring was done using a Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) monitor. Carotid-Femoral pulse wave velocity measurements were performed using a high fidelity SPC-301 micromanometer (Millar instruments Inc., Houston, TX).*

Results. *Of the 288 males, 140 were classified as non-dippers. Of the 508 females, 273 were classified as non-dippers. In the general population, males had higher triglycerides when compared with females 1.46±0.96 vs 1.13±1.02, p<0.0001. Additionally, dipper males had higher serum TRGL when compared with dipper females 1.32±0.98 vs 1.06±0.58, p = 0.0012. Non-dipper males also had higher serum TRGL when compared with non-dipper females 1.61±2.05 vs 1.19±1.14, p=0.0078. Serum HDLc was lower in the non-dipper male group when compared to the non-dipper female group (p=0.008). In both male and female groups, non-dippers had higher CFPWV when compared with dippers; 7.53±3.60 vs 5.74±2.47, p<0.0001 and 6.64±2.52 vs 5.98±2.23, p=0.0021 respectively. However, non-dipper males had significantly higher CFPWV when compared with non-dipper females (7.53±3.60 vs 6.64±2.52, p=0.0031).*

Conclusions. *Therapies targeting a reduction of serum triglycerides levels might be beneficial in improving arterial compliance with or without the presence of non-dipping.*

KEYWORDS: **Non-dipping; arterial stiffness; lipids.**

Introduction

A non-dipping blood pressure profile (NDP) is defined as failure of the night-time blood pressure (BP) values to drop by a minimum of 10% of the daytime values [1]. A non-dipping blood pressure profile (NDP) is associated with increased arterial stiffness and other cardiovascular target organ damage and is also a stronger predictive index of cardiovascular morbidity and mortality [2]. Arterial stiffness, which is one of the complications of non-dipping, is an established marker of arteriosclerosis and subclinical atherosclerosis and is an independent predictor of future adverse cardiovascular events [3]. Various techniques are available to assess arterial stiffness but some of these are inconvenient. Carotid-femoral pulse wave velocity (CF-PWV) is a simpler, practical and more reproducible way of evaluating arterial stiffness. It is considered the 'gold standard' for the measurement of arterial stiffness [4]. It has been proved that increase in arterial stiffness is an independent predictor of all causes of cardiovascular morbidity and mortality [5]. Cardiovascular risk factors such as age, diabetes and hypertension have been established to be positively associated with arterial stiffness [6]. However, the contribution of serum lipids to the development of arterial stiffness is not clear. Studies on the relationship between serum lipids and arterial stiffness have been conflicting [7, 8]. Additionally, there is no data investigating the possible role of serum lipid profile in non-dipping related increase in arterial stiffness. Thus, the study was focused on describing the serum lipid profile in non-dipping related increase in arterial stiffness measured by CF-PWV.

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Methods

Study Population

This study was approved by the Human Research Ethics Committee HREC (medical) of the University of the Witwatersrand Johannesburg South Africa (HREC approval number

M170213). This study was carried out according to the Helsinki declaration on ethical principles for medical research involving human subjects. The participants gave informed, written consents before the commencement of the study. Standard questionnaire was given to the participants to record data on medical history, smoking habits, alcohol intake, gender and age. The participants were randomly recruited from the SOWETO area of Johannesburg South Africa and were of black African origin. Of the 1,219 participants enrolled in this study only 796 had complete 24-hour ambulatory BP monitoring reports and their data was included in the statistical analysis. The minimum age of the participants was 18 years old.

Anthropometric measurements

Height, weight, waist and hip circumference were measured while the participants were in the standing position with no shoes on. Height was expressed in meters (m) while waist and hip circumferences were expressed in centimetres (cm). Body mass index (BMI) was expressed as kg/m^2.

Ambulatory blood pressure measurements

A twenty-four-hour ambulatory BP monitoring was carried out during the participants' typical working day or a day involving their usual activities using a Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) monitor. The monitor was programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and then 30-minute intervals from 22:01 to 05:59. Participants were instructed to note the time they go to bed and the time they wake up in the morning in an activity diary which was used to determine 'awake' and 'asleep' periods. Upon completion, data was transferred from the ambulatory BP monitors to a computer for analysis. A recording was considered as successful if at least 90% of valid recordings were obtained. Participants were classified as dippers if their nocturnal drop in 24-hour systolic BP was >10% and as non-dippers if they had a nocturnal drop in 24-hour systolic BP of <10%.

Carotid-Femoral Pulse Wave Velocity (CFPWV) measurement

Carotid-Femoral PWV measurements were conducted to determine the level of arterial stiffness. After resting for about 15 minutes in the supine position, CF-PWV was measured using a high fidelity SPC-301 micromanometer (Millar instruments Inc., Houston, TX) interfaced with a computer running SphygmoCor software version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Pulse pressure waveforms were recorded from the right common carotid artery and the right femoral artery. Pulse wave velocity was calculated automatically by the software.

Blood sample collection

Venous blood samples to determine serum lipid profiles were taken for the participants by an experienced qualified nurse who is part of the research group. The participants were asked to relax, and blood sample was collected in the sitting position. For plasma analyses, blood samples were collected into 9 ml EDTA Vacuette® (Greiner Bio One International GmbH) blood collection tubes. Blood samples were collected into 5ml Vacuette® Serum Gel tubes, gently inverted and allowed to clot for 30 minutes. The sample was then centrifuged at 2200 g for 15 minutes and the serum was carefully removed with a fine-bore pipette and then stored in properly labelled sterile vials and stored at 8 °C before pick-up by the laboratory. Samples were then picked up by the staff of CLS (Clinical Laboratory Services) at the National Health Laboratory Service (NHLS), South Africa, for analysis. All samples were taken to the laboratory within 7 days of blood collection.

Statistical analysis

All data were analysed using STATA (StataCorp LLC Texas USA) data analysis and statistical software version 13.0. Data were expressed as mean ± standard deviation (SD) for continuous variables. Categorical variables were expressed as absolute or relative frequencies or as percentages. A test for normality of continuous variables was assessed using Shapiro-Wilk's statistic or χ^2 test for categorical variables. Comparisons between dipper and non-dipper groups as well as gender groups were done using independent Student's t-test or one-way analysis of variance (ANOVA) (with post hoc Bonferroni tests). Simple regression was used to determine the relationship between carotid femoral pulse wave velocity and age in both genders. A P-value of <0.05 was considered as statistically significant.

Results

Demographic and clinical characteristics

The study population consisted of 796 participants and was classified according to gender and BP dipping status. There were a total of 288 males and 508 females. Of the 288 males, 148 were classified as dippers while 140 were classified as non-dippers. Of the 508 females, 235 were classified as dippers while 273 were classified as being non-dippers. In

both gender groups, non-dippers were older than dippers; 49.79±18.84 vs 39.06±17.88, p<0.0001 in the male group and 46.61±17.45 vs 41.25±17.33, p=0.0003 in the female group. Body mass index (BMI) was higher in females when compared to males in the general population 31.13±7.89 vs 24.96±5.14, p<0.0001. Additionally, females had higher BMI than males irrespective of dipping status. Waist circumference was higher in the female non-dipper group when compared with the male dipper group (88.23±15.22 vs 83.42±13.31, p=0.02) and also higher in the female non-dipper group when compared with the female dipper group (94.13±17.38 vs 89.50±15.35, p=0.0008). Females had higher waist circumference when compared with males irrespective of dipping status and gender group. There were more hypertensive participants in the non-dipper groups when compared with the dipper groups irrespective of gender. There were also more diabetic participants in the male non-dipper group when compared to the male dipper group. Males in the general population had higher triglycerides (TRGL) when compared with females in the general population 1.46±0.96 vs 1.13±1.02, p<0.0001. Additionally, dipper males had higher serum TRGL when compared with dipper females 1.32±0.98 vs 1.06±0.58 p=0.0012. Non-dipper males also had higher serum TRGL when compared with non-dipper females 1.61±2.05 vs 1.19±1.14, p=0.0078. Within the same gender group, serum triglycerides were not different between dipper and non-dippers. Serum HDLc was lower in males when compared with females in the general population (p<0.0001). Serum HDLc was also lower in the non-dipper male group when compared to the non-dipper female group (p=0.008). Females had higher serum LDLc when compared to males in the general population 2.72±0.89 vs 2.48±0.62, p<0.0001. Serum LDLc levels did not differ between dippers and non-dippers within gender groups; however, LDLc levels were higher in the female non-dipper group when compared with the male non-dipper group (p<0.05). There were no differences in other clinical and demographic characteristics. These results are presented in Table 1.

Carotid femoral pulse wave velocity (CFPWV) by gender and dipping status

In both male and female groups, non-dippers had higher CFPWV when compared with dippers; 7.53±3.60 (n=130) vs 5.74±2.47 (n=134), p<0.0001 and 6.64±2.52 (n=232) vs 5.98±2.23 (n=203), p=0.0021 respectively. Non-dipper males however had significantly higher CFPWV when compared with non-dipper females (7.53±3.60 vs 6.64±2.52, p=0.0031). These results are presented in Figure 1.

Relationship between age and carotid femoral pulse wave velocity (CFPWV)

Carotid Femoral Pulse Wave Velocity increased with age amongst males and females irrespective of dipping status. However, the age-related increase in CFPWV was steeper in males when compared to females (p<0.0001) irrespective of dipping status. The age-related increase in CFPWV was steeper in both non-dipper males and females when compared to dipper males and dipper females (p<0.0001). These results are presented in Figure 2.

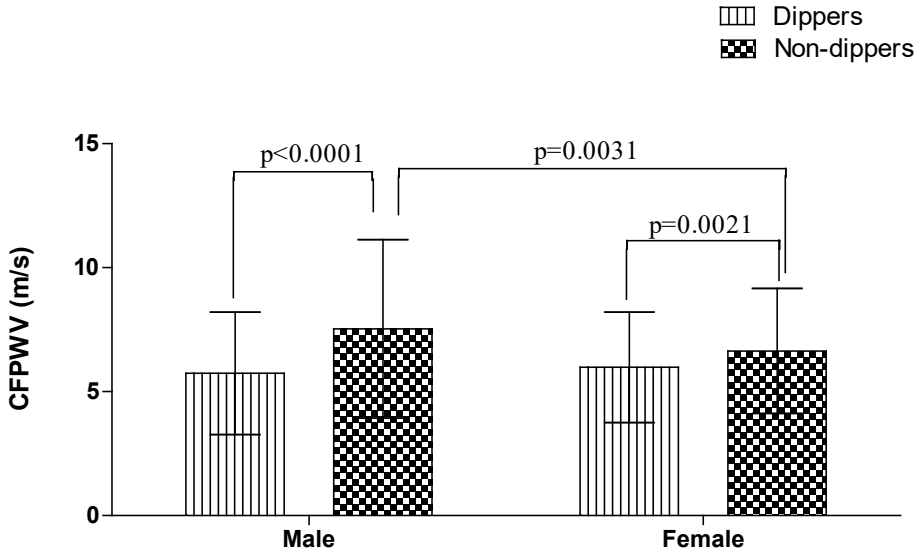

Fig. 1. Carotid femoral pulse wave velocity (CFPWV) of the participants according to blood pressure dipping status and gender. Results are presented as mean±SD.

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Table 1. Demographic and clinical characteristics of participants according to gender and blood pressure dipping status

	Males				Females			
	All	**Dippers**	**Non-Dippers**	**P**	**All**	**Dippers**	**Non-dippers**	**P**
N (%)	288 (36)	148 (51.40)	140 (48.61)		508 (64)	235 (46.25)	273 (53.74)	
Age (years)	44.2±19.09	39.06±17.88	49.79±18.84#	<0.0001*	44.14±17.58	41.25±17.33	46.61±17.45	0.0003*
BMI (kgm^{-2})	24.96±5.14£	24.32±4.92ϕ	25.63±5.30#	0.35	31.13±7.89	30.12±7.38	32.00±8.22	0.30
Waist circ (cm)	85.76±14.45£	83.42±13.31ϕ	88.23±15.22#	0.002*	91.98±16.62	89.50±15.35	94.13±17.38	0.0008*
Smokers (%)	36%	36%	31%		5%	6%	3%	
Alcohol use (%)	38%	40%	36%		11%	11%	10%	
Hypertensive (%)	8%	25%	50%		40%	33%	46%	
Diabetics (%)	10%	5%	14%		8%	6%	5%	
TCHOL (mmol/L)	4.47±1.06	4.45±1.05	4.48±0.99	0.401	4.67±1.02	4.59±1.03	4.57±1.31	0.425
TRGL (mmol/L)	1.46±0.96£	1.32±0.98ϕ	1.61±2.05#	0.06	1.13±1.03	1.06±0.58	1.19±1.14	0.06
HDLc (mmol/L)	1.35±0.42	1.40±0.53	1.30±0.43#	0.08	1.44±0.21	1.47±0.44	1.44±0.38	0.205
LDLc (mmol/L)	2.48±0.62£	2.45±0.87	2.50±0.87#	0.3131	2.72±0.89	2.63±0.94	2.79±1.06	0.091

*BMI = body mass index; Hip circ, hip circumference; Waist circ, waist circumference; TCHOL = Total cholesterol; TRGL = Triglycerides; HDLc = High density lipoprotein cholesterol; LDLc = Low density lipoprotein cholesterol. Data were presented as mean±SD or number (%). *A P value ≤0.05 depicts significant difference between dippers and non-dippers of same gender. # depicts significant difference (p<0.05) between non-dipper males and non-dipper females. ϕ depicts significant difference (p<0.05) between dipper males and dipper females. £ depicts significant difference (p<0.05) between males and females in the general population.*

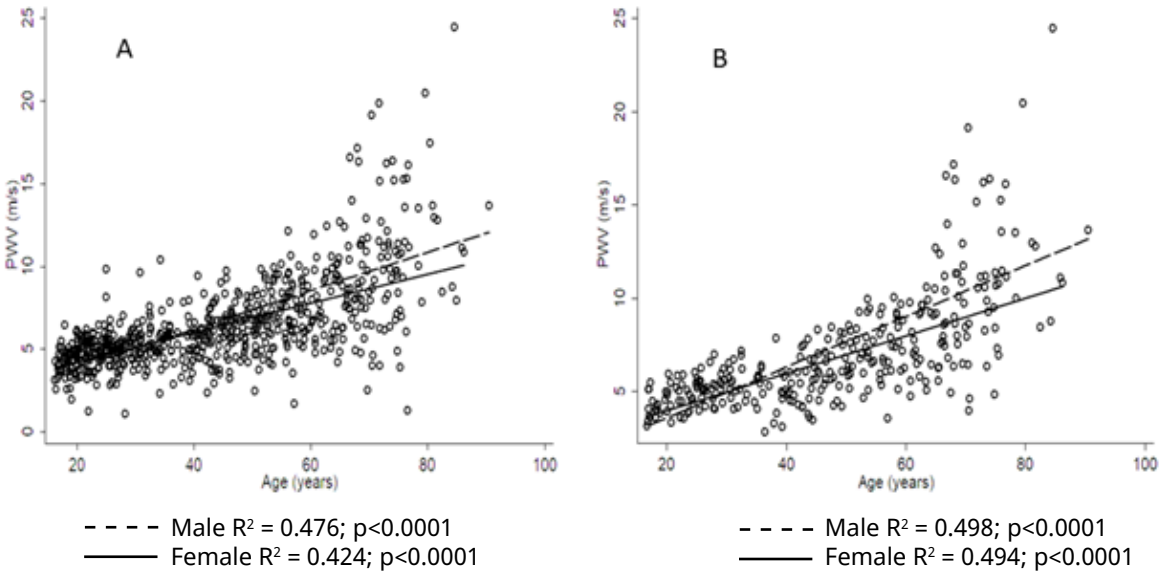

Fig. 2. Age related increase in pulse wave velocity. Panel A represents dippers population while panel B represents non-dippers. PWV = Pulse Wave Velocity.

Discussion

In this cross-sectional study of black South Africans, it was demonstrated that non-dippers have higher arterial stiffness as measured by CFPWV when compared with dippers. We also show that male non-dippers have greater arterial stiffness and greater age-related increase in arterial stiffness when compared with female non-dippers. Males had significantly higher serum triglycerides when compared with females irrespective of blood pressure dipping status. To our knowledge, this is the first time that serum lipid profiles are considered in non-dipping related arterial stiffness.

The males in our cohort had greater levels of triglycerides irrespective of dipping status irrespective of dipping status. We believe that serum triglycerides might explain the higher arterial stiffness observed in males. This is consistent with the findings of Wang and colleagues where they showed in a community-based study involving 1, 447 participants, that lower levels of triglycerides were significantly associated with decreases in CFPVW even after adjusting for confounding variables [9]. Zhao and colleagues also showed similar results [10]. They established that serum triglycerides had a positive relationship with brachial ankle pulse wave velocity (baPWV) in 1133 participants aged from 50 to 90.

The present study shows a significantly lower serum HDLc in non-dipper males when compared with non-dipper females. This might also explain the gender differences in arterial stiffness between non-dipper males and females. The role of HDLc in arterial stiffness has been studied and it has been suggested that low levels of HDLc is a risk factor for cardiovascular and cerebrovascular diseases [11]. Similarly, different studies have shown an inverse relationship between HDLc and pulse wave velocity, suggesting that higher levels of HDLc might be associated with an improved arterial distensibility [12]. Wang and colleagues in a cross-sectional study showed that in 15, 302 participants, HDLc had an inverse relationship with CFPWV [13]. Because of the large population of this study and similarity in technique used in the assessment of arterial stiffness, we are confident about the validity of our results. Of interest is also the study by Shen and colleagues where it is shown that high serum levels of HDLc are associated with a lower risk of arterial stiffness specifically in women older than 50 years of age but not in age matched men and not in individuals younger than 50 years old [14]. The exact mechanism behind the relationship between HDLc and CFPWV is not quite clear but it has been postulated that high HDLc levels are inversely proportional to advanced glycation end products levels (AGEs) [15] which stimulate inflammatory pathways and stress signaling and can damage elastin molecules in the vascular wall leading to arterial stiffness [16]. It has also been shown that HDLc has beneficial effects on the vascular wall via its anti-apoptotic, anti-inflammatory and anti-thrombotic properties [17].

Additionally, we also show in this study that female non-dippers had higher LDLc concen-

trations when compared with non-dipper males. Although high serum levels of LDLc have been attributed to higher degrees of arterial stiffness [18], this might not explain the observed gender differences in arterial stiffness in this study. This suggests that LDLc might be less important at determining arterial stiffness when compared with HDLc and triglycerides in this population. This assumption may be supported by trials that have shown significant residual cardiovascular risk even when LDLc levels have reached treatment target levels. This residual cardiovascular risk has been attributed to high triglycerides levels despite normal LDLc levels [19]. It has also been shown that levels of HDLc have better prognostic capabilities when compared to levels of LDLc [20].

The significantly higher pulse wave velocity in non-dipper males could have also been accounted for by higher prevalence of diabetes in this group. Alecu and colleagues showed in 207 participants a strong correlation between diabetes and arterial stiffness and a stronger relationship between pulse wave velocity and age in diabetic individuals when compared with non-diabetic individuals [21], although the non-dipping component was not considered. A relatively larger study with 1,415 participants showed higher prevalence of arterial stiffness amongst diabetic participants. They also showed a higher risk of increased arterial stiffness amongst diabetic individuals in a 5-year follow-up study [22].

The present study shows an increase in CFPWV with age irrespective of gender, with males having a steeper increase in age related arterial stiffness when compared to females irrespective of dipper status. These observations are consistent with those of Alghathrif and colleagues where they showed in a population of 777 individuals with 354 men and 423 women, that men had a steeper longitudinal increase in CFPWV with advancing age when compared with women, which makes the males to have higher CPFPWV values after their fifth decade [23]. Our results are also in line with another study by Wen and colleagues [24] in which arterial stiffness was assessed by cardio-ankle vascular index (CAVI) in 18, 336 subjects and it was observed that aging led to a higher arterial stiffness in men when compared with women. Magalhães and colleagues also showed that males above 50 years old had a greater pulse wave velocity when compared to age matched females [25]. However, in contrast to our findings there are observations by Nethononda and colleagues where it was evidenced that females showed a steeper increase in aortic stiffness with age when compared with males [26]. This study consisted of 777 individuals with 408 females and 369 males. The pulse wave velocity was assessed using cardiovascular magnetic resonance. Similarly, a study by Tomiyama and colleagues reported that age induced arterial stiffness as measured by brachial ankle pulse wave velocity was higher in females when compared to males. This study involved 7,881 subjects with 4,488 males and 3,393 females [27]. The differences in observations between the latter mentioned studies are not quite clear, but despite their large sample sizes, they employed different methods to assess arterial stiffness. All the studies mentioned above have been conducted in general populations and the nocturnal BP dipping statuses of the participants were not taken into consideration. The reports that studied the non-dipping BP pattern alongside arterial stiffness did not consider age and gender differences [28]. Our results suggest that non-dipping related arterial stiffness is more severe in males when compared to females causing them to have a higher non-dipping related arterial stiffness and a steeper increase in age related arterial stiffness when compared with females. Aging is associated with increased sympathetic activation [29] and may lead to inflammation. Inflammation has been shown to induce arterial stiffness [30].

Our study population was modest in size and consisted entirely of black Africans living in Africa, this might limit the generalizability of our findings. Also, our study was cross-sectional, therefore, cause-effect relationships cannot be established.

Conclusions

The nocturnal non-dipping blood pressure profile is associated with higher arterial stiffness and a steeper age-related increase in arterial stiffness irrespective of gender. Non-dipper males have higher level of arterial stiffness and age-related increase in arterial stiffness when compared with non-dipper females. Therapies targeting a reduction of serum triglycerides levels might be beneficial in improving arterial compliance with or without the presence of non-dipping.

Conflict of interest

The authors report no conflict of interests in this study

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Author's contribution

Abdulraheem B. Bawa-Allah – conceptualization, formal analysis, investigation, methodology, project administration, software, validation, visualization, writing-original draft, writing-review and editing; *Mercy M. Mashao* – investigation, methodology, project administration; *Thamsanqa F. Nyundu* – investigation, methodology, project administration; *Edgar M. Phukubje* – investigation, methodology, project administration; *Brian G. Nkosi* – investigation, methodology, project administration; *Mandisa V. Ngema* – investigation, methodology, project administration; *Bongubuhle W. Mlambo* – investigation, methodology, project administration; *Muzi J. Maseko* – conceptualization, investigation, methodology, project administration, validation, visualization, writing- review and editing, supervision.

ЛІПІДНИЙ СПЕКТР КРОВІ ТА АРТЕРІАЛЬНА ЖОРСТКІСТЬ У ПАЦІЄНТІВ З NON-DIPPER-ДОБОВИМ ПРОФІЛЕМ АРТЕРІАЛЬНОГО ТИСКУ

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Вступ. *Добовий профіль артеріального тиску (АТ) non-dipper, який супроводжується недостатнім зниженням АТ вночі, асоціюється з підвищеною жорсткістю артерій та ураженням органів-мішеней. Ліпідний профіль – одна з важливих детермінант артеріальної жорсткості.*

Мета роботи. *Мета дослідження – оцінити взаємозв'язки між ліпідним спектром крові та артеріальною жорсткістю у пацієнтів з добовим профілем АТ non-dipper.*

Методи. *Було проведене поперечне (одномоментне) дослідження 796 (288 чоловіків та 508 жінок) осіб-африканців. 24-годинний добовий моніторинг АТ проводили з використанням обладнання Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA). Швидкість поширення пульсової хвилі по артеріях еластичного типу (ШППХ) визначали за допомогою мікроманометра SPC-301 (Millar instruments Inc., Houston, TX).*

Результати. *Серед 288 чоловіків у 140 добовий профіль АТ був типу non-dipper. Серед 508 жінок цей тип був у 273. У загальній когорті рівень тригліцеридів був вищим у чоловіків порівняно з жінками 1,46±0,96 vs 1,13±1,02, p<0,0001. Окрім того, у чоловіків з добовим профілем АТ dipper рівень тригліцеридів був вищим відносно жінок-dippers 1,32±0,98 vs 1,06±0,58 (p=0,0012) відповідно. Non-dippers-чоловіки мали вищий рівень тригліцеридів також відносно і жінок-non-dippers: 1,61±2,05 vs 1,19±1,14 (p=0,0078) відповідно. Рівень ліпопротеїдів високої щільності був достовірно нижчим у чоловіків-non-dippers порівняно з жінками з групи non-dippers (p=0.008). У обох статей і чоловіків і жінок non-dippers ШППХ була вищою у порівнянні з dippers; 7,53±3,60 vs 5,74±2,47, p<0,0001 та 6,64±2,52 vs 5,98±2,23 (p=0,0021) відповідно. Однак у чоловіків non-dipper ШППХ була достовірно вищою порівняно з аналогічною групою жінок (7,53±3,60 vs 6,64±2,52, p=0,0031).*

Висновки. *Враховуючи отримані нами дані, фармакотерапія спрямована на зниження рівня тригліцеридів у сироватці крові може впливати на перебіг артеріальної гіпертензії та її наслідки незалежно від типу добового профілю артеріального тиску.*

КЛЮЧОВІ СЛОВА: **Non-dipping профіль артеріального тиску; артеріальна жорсткість; ліпідний профіль крові.**

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DISSEMINATED HISTOPLASMOSIS LEADING TO HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN AN IMMUNOCOMPETENT PATIENT (case report)

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Background*: Emerging fungal infections can pose a serious threat in contemporary healthcare due to host variations, clinical presentation and emerging resistance. Histoplasma capsulatum is a thermally dimorphic fungus, which acts as a Trojan horse by residing inside macrophages. Histoplasmosis is an emerging infection and its association with hemophagocytic lymphohistiocytosis (HLH) in immunocompetent patients has been scantily reported in the literature.*

Objective. *The aim of the study was to explore disseminated histoplasmosis with the help of case report.*

Methods*: A case report of histoid leprosy is presented.*

Results*: A male patient of 47 yearsof age, under treatment for chronic obstructive pulmonary disease for five years and diabetes mellitus Type-II for two years, presented with fever of unknown origin (FUO) with evidence of HLH in the bone marrow. Core biopsy of the liver and spleen showed a dense tissue infiltrate with vacuolated histiocytes containing Histoplasma capsulatum, eosinophils, some lymphocytes and plasma cells.*

Conclusion*: Histoid leprosy is a discrete infrequent form of multibacillary leprosy with distinctive clinical, bacteriological and histomorphological features. Histopathologic examination with modified fite stain remains the mainstay of diagnosis.*

KEY WORDS: **histoplasmosis; hemophagocytic lymphohistiocytosis.**

Introduction

Histoplasma capsulatum is a thermally dimorphic fungus, which acts as a Trojan Horse by residing inside macrophages [1]. Most individuals with intact cellular immunity are asymptomatic. A patient presented with fever of unknown origin (FUO) with evidence of hemophagocytic lymphohistiocytosis (HLH) in the bone marrow. Emerging fungal infections can pose a serious threat in contemporary healthcare due to host variations, clinical presentation and emerging resistance [2-8]. There are diagnostic stringencies in resource limited facilities [9, 10]. Histoplasmosis is an emerging infection and its association with HLH in immunocompetent patients has been scantily reported in the literature [11-16].

Case Report

A male patient of 47 years old, under treatment for chronic obstructive pulmonary disease for five years and diabetes mellitus Type-II for two years, presented with rashes around knee for 6 months, fever for 2 weeks and black stools for 3 days. Pallor, pedal edema, hepatomegaly 7 cm below right subcostal margin and splenomegaly 9 cm below left subcostal margin were evidenced. Investigated for FUO, hemoglobin was 5.5-8.3 gm/dl, total leucocytes 3600-4200/cumm with normal differential, serum ferritin was 306 ng/dl, while other tests were non-contributory towards diagnosis. CT thorax and abdomen revealed hepatosplenomegaly, retroperitoneal lymphadenopathy with mild ascites and solitary lesion in spleen. Colonoscopy was non-contributory. Bone marrow aspirate and biopsy showed evidence of HLH (Fig. 1).

While on treatment, the patient succumbed to his illness. Consented post-mortem needle biopsy from liver, spleen, lungs and kidneys was done. Core biopsy of the liver and spleen showed a dense tissue infiltrate with vacuolated histiocytes containing histoplasma capsulatum, eosinophils, some lymphocytes and plasma cells.

Discussion

Histoplasmosis is an endemic infection in most of the USA, Asia and Africa caused by

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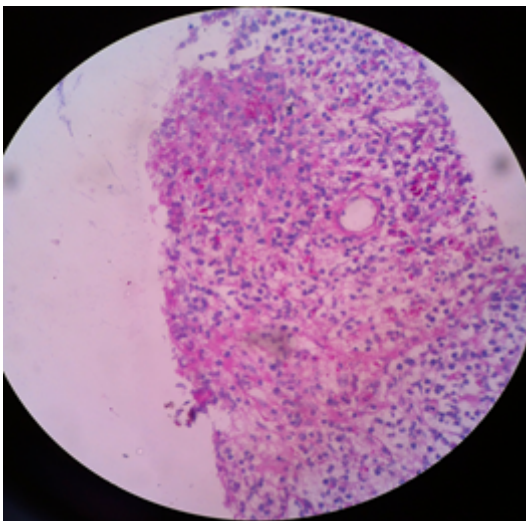

Fig. 1. Photomicrograph: HPE 400X; Bone marrow aspirate with Histoplasma, increased histiocytes and evidence of hemophagocytosis.

infectious bat and birdexcretions. Disseminated histoplasmosis, classically described in the immunocompromised, can occur in immunocompetent patients. Clinical presentations vary depending on the size of the inoculum, host's immune status and presence of underlying lung disease. Overt symptoms occur in 5% healthy individuals after low-level exposure; however 75% may get affected with heavy exposure. Mostly asymptomatic, mild flu-like illness, fever, chills, sweating, cough, chest and joint pain may occur. Acute histoplasmosis may last 1-5 days whereas chronic histoplasmosis, mostly associated with lung infections, can last 10-21 days and is associated with weight loss, persistent fatigue and night sweats. Associated acute or subacute pulmonary disease, progressive disseminated disease, pericarditis, arthritis, mediastinitis, hepatomegaly, splenomegaly and bone marrow suppression may occur [17-19]. The patient presented with fever, fatigue, polyarthralgia, skin rashes and black stools with underlying chronic obstructive pulmonary disease. Along with anemia and hepatosplenomegaly, the clinical picture fits histoplasmosis [11-16].

HLH is an overwhelming inflammatory response, resulting in cytokine storm and activation of monocyte-macrophage system resulting in multiorgan dysfunction. It is a secondary phenomenon to infections, malignancies, autoimmune disorders and drug reaction. HLH is associated with high mortality although successful treatment has been registered [11,14,15].

Diagnosis of histoplasmosis involves staining, isolation, serology and antigen detection. Bone marrow gives the highest diagnostic yield. Antigen detection in urine and serum by radioimmunoassay is useful in an immunocompromised patient when antibody production may be impaired. Other laboratory abnormalities include anemia, leukopenia, pancytopenia, elevated liver enzymes, increased ferritin and lactate dehydrogenase. Our diagnosis was based on clinical presentation and histopathology of liver and spleen [6], when culture was negative.

A high index of suspicion is required as 100% mortality seen in untreated histoplasmosis, can fall to 70% when adequately treated with Amphotericin-B [4, 20-25].

Risk factors for acquiring acute or chronic histoplasmosis in immunocompetent patients are for farmers and travelers having prolonged contact with rural or endemic environment, speleologists/spelunkers coming in contact with bat guano, and farmers coming in contact with soil enriched with bird guano. Heavy infective inoculum leads to acute presentation whereas low inoculum may lead to asymptomatic or chronic infection. Prolonged exposure to endemic area in Brazil led to histoplasmosis in 43.9-82.9% of immunocompetent patients [26-29].

Conclusion

Disseminated histoplasmosis needs to be differentiated from common diseases like tuberculosis, lymphoma or metastatic malignancy. Emerging opportunistic resistant infections warrant a high degree of clinical intuition and mental mobility for optimal management.

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Conflict of Interests

The authors declare no conflict of interest.

Author's Contributions

I.D. Khan – study concept/design, conduct of study, drafting and manuscript revision, final approval of manuscript; *M. Brijwal* – study concept/design, final approval of manuscript; *I. Joshi* – study concept/design, final approval of manuscript; *B. Singh* – conduct of study; *B. Poonia, G. Gonimadatala, S. Mangalesh, A. Yadav* – statistical analysis, drafting and manuscript revision; *H. Rajput* – drafting and manuscript revision, *N. Bhuttay* – drafting and manuscript revision.

ДИСЕМІНОВАНИЙ ГІСТОПЛАЗМОЗ ЯК ЕТІОЛОГІЧНИЙ ЧИННИК ГЕМОФАГОЦИТАРНОГО ЛІМФОГІСТІОЦИТОЗУ У ІМУНОКОМПЕТЕНТНОГО ПАЦІЄНТА (клінічний випадок)

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Вступ. *Зростання кількості та частоти грибкових інфекцій може становити серйозну загрозу сучасній системі охорони здоров'я через варіативність клінічного перебігу, симптомів та ознак захворювання, резистентність до лікування. Histoplasma capsulatum – це диморфний гриб, який поводиться як «троянський кінь» проникаючи всередину макрофагів. Гістоплазмоз – захворювання, що останнім часом все частіше діагностується, однак про його взаємозв'язок з гемофагоцитарним лімфогістіоцитозом у імунокомпетентних осіб надзвичайно мало інформації.*

Мета роботи *на прикладі клінічного випадку дослідити перебіг дисемінованого гістоплазмозу.*

Методи. *Описано клінічний випадок гістоїдної лепри у пацієнта.*

Результати. *Чоловік, 47 років, котрий останніх п'ять років лікувався від ХОЗЛ та цукрового діабету другого типу, звернувся до лікаря зі скаргами на лихоманку невідомого генезу. Було діагностовано гемофагоцитарний лімфогістіоцитоз кісткового мозку. На препаратах печінки та селезінки посмертної біопсії органів знайдено щільний тканинний інфільтрат з вакуолізованими гістіоцитами, які містили Histoplasma capsulatum, еозинофіли, невелику кількість лімфоцитів та плазмоцитів.*

Висновки. *Гістоїдна лепра – рідкісна форма мультибацилярної лепри з характерними клінічними, бактеріологічними та гістомофологічними ознаками. Проведення гістологічного дослідження дозволяє діагностувати захворювання.*

КЛЮЧОВІ СЛОВА: **гістоплазмоз; гемофагоцитарний лімфогістіоцитоз.**

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IMPACT OF MOLECULAR METHOD FOR THE DIAGNOSIS OF ACUTE BACTERIAL MENINGITIS IN A TERTIARY HEALTH CARE CENTRE IN NORTH INDIA

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Background. *Acute bacterial meningitis is one of the significant infectious diseases that add an immense burden to the health system. Proper management of meningitis is an invincible need to overcome the severe consequences.*

Objectives. *The aim of the study is detection of the etiological agents of acute bacterial meningitis by PCR.*

Methods. *Total 267 CSF samples collected from suspected bacterial meningitis cases were processed for the detection of S. pneumoniae, H. influenzae, N. meningitidis, E. coli and Group B streptococci by conventional and molecular diagnosis method. CSF was inoculated on Blood, chocolate and MacConkey agar plates and incubated at 37 °C for 24-48 hrs. Bacteria grown were identified by Matrix assisted laser desorption/ionization time of flight (MALDI-TOF). Multiplex PCR of the enlisted bacteria was performed using DNA extracted from CSF by DNA extraction kit (Qiagen, USA).*

Results. *5 (1.87%) out of the total 267 CSF samples were culture positive (3 S. pneumoniae and 2 E. coli) and 28 (10.49%) had detectable DNA by conventional PCR. Out of these 28 samples, 20 (71.43%) were S. pneumoniae and 8 (28.57%) were E. coli. 15 (53.57%) out of total S. pneumoniae were present in children below 5 years of age. Similarly, E. coli was predominant in neonates. Sensitivity and specificity of the PCR was 100% and 95-98% respectively.*

Conclusions. *Streptococcus pneumoniae is the commonest cause of community acquired bacterial meningitis in children below five years of age. Hence, for the promising outcome, PCR should be implemented for the diagnosis of acute bacterial meningitis.*

KEY WORDS: **cerebrospinal fluid (CSF); acute bacterial meningitis; PCR; MALDI-TOF.**

Introduction

Bacterial meningitis is one of the dangerous infectious diseases of central nervous system (CNS). Meningitis is an inflammation of delicate membrane known as meninges covering brain and spinal cord and marked by intense headache, fever and nuchal rigidity. Despite advances in treatment, acute bacterial meningitis (ABM) is the major cause of mortality and morbidity in developed countries as well as in developing ones [1]. In India and other developing countries mortality rate varies 16-32% [2-5]. Individuals with suspected meningitis require prompt diagnosis and treatment. Delay in management can result in poor outcome of the disease [6].

The most common bacteria causing ABM are *Streptococcus pneumoniae, Haemophilus influenza*e, *Neisseria meningitidis*, *Listeria monocytogenes*, Group B *Streptococci* and *E. coli* [7]. Etiological agents of ABM vary globally depending on age, geographical area and immune status [8, 9]. Hence, there is need for regularly reviewing of bacterial meningitis.

Culture is considered as the 'Gold standard' for the diagnosis of acute bacterial meningitis as well as equally important for antibiotic susceptibility testing of the causative agents that account for treatment [10]. However, it takes longer for identification of the pathogens and its outcome is influenced by intake of antibiotics prior to lumber puncture and number of bacteria (CFU/ml) present in the CSF. To overcome this diagnostic glitch, polymerase chain reaction (PCR) is gaining momentum for a rapid diagnosis of ABM [11-17]. Hence, this experiment have been designed and performed to detect the etiological agents of community acquired bacterial meningitis by molecular method as PCR does not rely on viability of the organism.

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Methods

A perspective study was designed and carried out at the Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India. Total 267 cerebrospinal fluids (CSF) samples from suspected acute bacterial meningitis cases were collected in March, 2015 – April, 2018. All samples were processed for cytological, biochemical, Microbiological (Gram stain, Latex agglutination and culture) and molecular examination. CSF was centrifuged at 10,000 rpm for 10 minutes. Sediment was used for Gram stain and culture. Blood, chocolate and Mac-conkey agar plates were used for growth of the bacteria. After inoculation, the plates were incubated at 37 °C for 24-48 hrs with 5% CO_2. Isolates grown on the culture plates were identified by MALDI-TOF (Vitek MS Biomerieux, France). The isolates were further processed for antibiotic susceptibility tests. Nosocomial infection was defined as a positive bacterial infection that was not present at the time of hospital admission or clinical evidence of an infection no sooner than 48 hours after admission. Remaining patients were considered to have community acquired infection.

Identification of isolates by MALDI-TOF. For identification of the bacteria, a single bacterial colony was taken, and the smear was made on the MALDI-TOF slide with the help of wooden stick and 0.5 µl matrix (α-cyano-4-hydroxycinnamic acid) was added. The slide was kept in air at a room temperature for 5 minutes to dry and finally slide was kept in the MALDI-TOF machine (VITEK MS Biomerieux, France) for acquisition and identification. The isolates identified were further processed for antibiotic susceptibility test.

Antibiotics susceptibility test. Mueller Hinton Agar was used to perform an antibiotic susceptibility test. The isolate was inoculated in 0.5% peptone water and incubated at 37 °C for 2 hrs. Turbidity was compared with 0.5 McFarland solutions so that bacterial count to be >10^5 CFU/ml. Antibiotics used were amikacin (10 µg), co-trimoxazole (25 µg), cephotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (30 µg), erythromycin (30 µg), penicillin G (10units), piperacillin + tazobactam (100/10 µg), imipenem (10 µg), Netilmicin (30 µg), chloramphenicol (30 µg) and vancomycin (30 µg). The antibiotic discs were obtained from Hi Media Laboratories, Mumbai, India. Antibiotic susceptibility results were interpreted as per CLSI guideline. The *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC25923 strains were used for quality control.

Latex agglutination test (LAT). A supernatant of the centrifuged CSF was used for detection of soluble bacterial antigen of *Streptococcus pneumoniae, Neisseria meningitidis* A, B, C, and W135, *Streptococcus agalactiae*, *Escherichia coli*, and *Haemophilus influenzae* type B by latex agglutination test (LAT) following manufacturer's instructions. The Directigen™ Meningitis combo Kit (BD, USA) was used for the LAT.

Extraction of DNA. Extraction of DNA from CSF samples was carried out using the Mini DNA extraction Kit (Qiagen, USA) following manufacturer's instructions. DNA was eluted in 100 µl elution buffer and stored at -20 °C for future use in the experiment.

Primer design and PCR conditions. For detection of *S. pneumoniae*, *H. influenzae* and *N. meningitidis* semi-nested multiplex PCR was designed. However, for *E. coli* & Group B *streptococcus the* Multiplex PCR was done. Primers were designed accordingly that is presented in Table 1.

Table1. Sequence of primers used in this study

Organisms	Target Gene	Primer	Amplicon length (bp)	Reference
Seminested Multiplex PCR				
Universal Primer	U3 (16S rRNA)	5'-GTG CCT GCA GCC GCG GTA AT-3'	1000	Chakrabarti P et al, 2009 [18]
	U8 (16S rRNA)	5'-AAG GAG GGG TGT GTAC-3'		
S. pneumoniae	ply	5'-GTA CAA CGA GTC GCA AGC-3'	293	
H. influenzae	bex	5'-CCT AAG AAG AGA TCG AG-3'	543	
N. meningitidis	ctr	5'-TGT TGC GCA ACC TGA TTG-3'	710	
Multiplex PCR				
E. coli	16S rRNA	5'-TGC CTG ATG GAG GGG GAT AA-3'	776	This study
		5'-TTT AAC CTT GCG GCC GTA CT-3'		
S. agalactiae	sip	5'-ACA ACG GAA GGC GCT ACT GTT C-3'	255	Bergh K et al, 2004 [19]
		5'-ACC TGG TGT TTG ACC TGA ACT A-3'		

Amplification of DNA extracted from the CSF. The PCR mixture (25 µl) contained 1×PCR buffer 2.5 µl with 2.5 mM $Mgcl_2$, 0.5 µl dNTPs (10mM each), 0.25 µl Taq polymerase (Promega, 500U) & Primer 0.2 µl (10 µM each) for semi-nested multiplex PCR. However, for multiplex PCR, primer concentration used was 1 µl (10 µM each) and 2.5 µl of the target DNA. Finally, the volume of the PCR mixture was adjusted by adding milli Q water. PCR was performed in the Thermocycler (Agilent Technologies, Surecycler 8800, USA) with the following cycling condition for multiplex PCR, i.e. initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 1 min, Annealing 55 °C for 30 sec, extension 72 °C for 30 sec and final extension at 72 °C for 10 min with 4 °C holding temperature, the number of cycles was 35. After amplification of the bacterial DNA, gel electrophoresis of the PCR product was run for 30 minutes in 1.5% agarose with ethidium bromide and visualize under UV fluorescence to check the product size. Representative Image of the Gel electrophoresis is presented in Fig. 1.

Statistical analysis. of the tests was performed by the Wilcoxon Singed-rank test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were evaluated.

Ethical Approval. The study was approved by the Institute Ethics Committee of All India Institute of Medical Sciences, New Delhi (No: IESC/T-20)

Results

The finding of the study was interpreted and correlated with the etiological agents of ABM in all age group (0 days-84 years) with conventional and molecular techniques. Out of total 267 cases of suspected meningitis patients, 194 (72.60%) were male and 73 (27.34%) were female. The main clinical feature shown by the patients were fever 258 (96.63%), headache 210 (78.65%) and altered sensorium 181 (67.79%) details are given in Table 2.

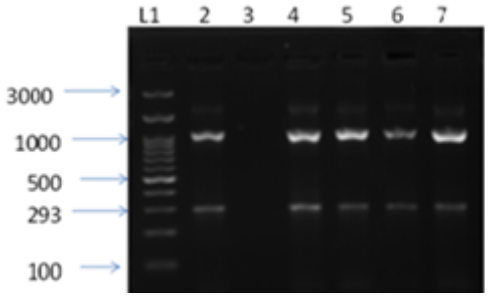

Fig.1. A gel image of PCR product of *S. pneumoniae* in 1.5% agar.
L_1 = Ladder, L_2 = Positive control, L_3 = Negative control & $L_{4,5,6,7}$ = CSF sample.

The CSF glucose and protein level of the majority of the patients were <45 mg/dl and >55 mg/dl respectively. Out of 267 CSF samples, 5 (1.87%) were proved to be positive by culture. Among these 5 isolates, 3 (60%) were *S. pneumoniae* and 2 (40%) were *E. coli.* Similarly, total 28 (10.49%) bacteria were detected by conventional PCR (CPCR) as the culture positive bacteria were also positive by PCR; out of 23 bacteria detected by PCR only, 6 (26.09%) were *E. coli* and 17 (73.91%) were *S. pneumoniae*. Main cases of the organisms revealed were of the age group of 0-5 yearsold. Likewise, 4 (50%) cases of *E. coli* were revealed in neonates. In case of *S. pneumoniae,* 15 (53.57%) isolates were revealed in children below 5 years of age. The details of distribution of bacteria revealed are presented in Table 3.

Latex agglutination test (LAT) was used to detect the bacterial soluble antigen of the common etiological agents of acute bacterial meningitis: *Streptococcus pneumoniae, Neisseria meningitidis* A, B, C & W135, *Streptococcus agalactiae*, *Escherichia coli*, and *Haemophilus influenzae* type B. However, only *S. pneumoniae* and *E. coli* was detected by the LAT in this study. The LAT has detected more organisms than the culture. Total 15 isolates were detected by the LAT. Out of 15 LAT positive isolates 10 (5 *S. pneumoniae* and 5 *E. coli*) were positive by the LAT only. However,

Table 2. Demographic details of the meningitis patients

Clinical Features	Number of patients
Fever	258 (96.63%)
Headache	210 (78.65%)
Nuchal rigidity	57 (21.34%)
Altered sensorium	181 (67.79%)
Nausea/Vomiting	54 (20.24%)
Kernig's and Brudzinski's sign	51 (19.10%)
Bulging anterior fontanelle	32 (11.98%)

Table 3. Age wise distribution of the isolates detected from CSF

Isolates	Methods of detection	Age group						Total
		Neonates	1-3 months	3-24 months	2-5 yrs	5-50 yrs	>50 yrs	
E. coli	Culture	01	–	–	–	–	01	02
	CPCR	03	02	–	–	–	01	06
S. pneumoniae	Culture	–	–	01	01	01	–	03
	CPCR	–	02	07	04	02	02	17
Total		04	04	08	05	03	04	28

3 *S. pneumoniae* and 2 *E. coli* that were culture positive were also positive by the LAT.

Taking culture as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value were evaluated. Sensitivity and specificity of CPCR for *S. pneumoniae* and *E. coli* were found in 100% and 95-98% cases, respectively, with 95% CI that is presented in Table 4.

The antibiotic susceptibility test was performed for *E. coli* and *S. pneumoniae* detected from CSF by culture. Most of the antibiotics tested were susceptible to *S. pneumoniae* (100%). One (33.36%) *S. pneumoniae* showed resistance to co-trimoxazole. For *E. coli,* the most susceptible antibiotic was amikacin (100%) followed by colistin (100%) and imipenem (100%).

Table 4. Sensitivity, specificity, PPV, NPV of CPCR comparing with culture

Statistics	Conventional PCR			
	E. coli (N=6)		*S. pneumoniae* (N=17)	
	Value	with 95% CI	Value	with 95% CI
Sensitivity	100%	15.81% – 100%	100%	28.24% – 100%
Specificity	98.28%	96.29% – 99.36%	95.26%	95.53% – 97.22%
PPV	25%	13.10% – 42.42%	15%	9.99% – 21.92%
NPV	100%	–	–	95.30%
Accuracy	98.29%	96.31% – 99.37%	95.30%	92.59% – 97.24%

Discussion

Community acquired acute bacterial meningitis is an urgent health issue as most of the infection occurs in children below 5 years of age. In this study, total 267 CSF samples were included irrespective of the age limit. Of the total number of CSF, only 5 (1.87%) were found positive by culture for different etiological agents of community acquired bacterial meningitis. A study conducted by Zahra B. H et al. has shown culture positivity 2.5% [20]. Positivity of culture decreases 60-70% in case of CSF collected after administration of antibiotics [21]. In our study, low culture positivity may be due to intake of antibiotics prior to lumber puncture as our hospital is a referral center where majority of the patients admitted had already been administered with various antibiotics before getting admitted.

We detected 28 (10.14%) positive CSF by CPCR which were almost 10% more than the culture positives. Our finding is concurrent with the finding (11.76%) shown by Nour M et al. [22] but less than the finding by Zahra B. H. et al. and Chokrabarty P. et al. who have reported 15% and 19.8% positivity by mPCR respectively [18, 20]. Low positivity may be due to difference in sample size and organisms detected. Sensitivity and specificity of convention PCR was found 100% and 95-98% respectively for *E. coli* and *S. pneumoniae* with 95% CI. Finding was concurrent with the findings of Wu et al. [23].

In children below 1-24 months of age *S. pneumoniae* 10 (50%) was the main organism. However, *E. coli* 4 (50%) were found in neonates that was also a significant finding as *E. coli* and *S. agalactiae* are the major pathogens of the neonates [24, 25]. We have detected total 20 *S. pneumoniae*, 15 (75%) of which were in those less than 5 years of age. Likewise, out of these 15 cases, 8 (53.3%) were detected in those 3-24 months of age. More number of *S. pneumoniae* in children below 5 years of age was reported from India [26, 27]. However, 1 previous study conducted in 1999 by ISIB has reported *H. influenzae* as the main cause of bacterial menin-

gitis prior to the introduction of pentavalent vaccines [28].

In our study, only *E. coli* and *S. pneumoniae* were detected among the etiological agents of community acquired acute bacterial meningitis that may be a result of implementation of the Hib vaccination in national immunization program in India. *H. influenzae* detection rate has decreased drastically in recent times. Similarly, in India, incidence of *N. meningitidis* increased greatly during the epidemics and the epidemic cycle of *N. meningitidis* is at an interval of 15-20 years. Last episode of *N. meningitidis* epidemics was in 2005 [29].

Antibiotic susceptibility test result showed that most of the antibiotics used were susceptible to S. *pneumoniae*. One (33.3%) *S. pneumoniae* out of 3 was resistance to co-trimoxazole. However, no penicillin resistance *S. pneumoniae* was found. Similarly, amikacin, colistin and imipenem were the most susceptible (100%) antibiotics for *E. coli* though ciprofloxacin has shown (50%) resistance followed by cefo + salbactam (50%).

Conclusions

Acute bacterial meningitis is a severe medical emergency and *S. pneumoniae* is still the a major cause of community acquired bacterial meningitis in children. Molecular diagnosis is gaining momentum for a rapid detection of etiological agents with high sensitivity and specificity since it is unaffected by viability of the organisms. Hence, PCR should be implemented in the diagnosis to overcome the dependency on conventional method.

Conflict of Interest

The authors declare no conflict of interest.

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МОЛЕКУЛЯРНІ МЕТОДИ ДІАГНОСТИКИ ГОСТРОГО БАКТЕРІАЛЬНОГО МЕНІНГІТУ НА ТРЕТИННОМУ РІВНІ НАДАННЯ МЕДИЧНОЇ ДОПОМОГИ У ЛІКАРНЯХ ПІВНІЧНОЇ ІНДІЇ

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Вступ. *Гострий бактеріальний менінгіт – одне з найбільш небезпечних інфекційних захворювань, що слугує додатковим тягарем для системи охорони здоров'я. Відповідне лікування – необхідна умова для попередження та лікування тяжких наслідків хвороби.*

Мета дослідження – *встановлення етіологічних чинників гострого бактеріального менінгіту за допомогою ПЛР.*

Методи. *Загалом було відібрано 276 зразків спинномозкової рідини (СМР) від пацієнтів з підозрою на бактеріальний менінгіт для визначення його основних збудників S. pneumoniae, H. influenzae, N. meningitidis, E. coli та стрептококів групи B типовим способом та за допомогою методів молекулярної діагностики. Для проведення культурального дослідження зразків СМР використовували живильні середовища: кров'яний агар, шоколадний агар, середовище МакКонкі. Зразки інкубували при 37°C протягом 24-48 годин. Ріст бактерій досліджували методом мас-спектрометрії з лазерною десорбцією/ іонізацією MALDI-TOF. Мультиплексна ПЛР зразків СМР проводилася з використанням наборів для екстракції ДНК Qiagen (USA).*

Результати. *Із 276 досліджених зразків СМР, п'ять (1.87%) були культуро-позитивними (три – S. pneumoniae та два зразки – E. coli). За допомогою типової ПЛР ДНК збудника було знайдено у 28 зразках (10.49%). З цих 28 зразків у 20 (71.43%) – були ідентифіковані S. pneumoniae, а у 8 зразках (28.57%) – E. coli.*

Серед зразків, де було виявлено S. pneumoniae, 15 (53.57%) належали дітям до 5 років. E. coli переважала у новонароджених. Чутливість та специфічність ПЛР була 100% та 95-98% відповідно.

Висновки. *Streptococcus pneumoniae – найбільш поширений збудник бактеріального менінгіту у дітей віком до 5 років. Враховуючи результати наших досліджень, ПЛР-діагностика повинна впроваджуватися у комплекс методів діагностики гострого бактеріального менінгіту.*

КЛЮЧОВІ СЛОВА: **спинномозкова рідина (СМР); гострий бактеріальний менінгіт; ПЛР; MALDI-TOF.**

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SYSTEMIC OXIDATIVE STRESS AS A SURROGATE OF CORONARY ATHEROSCLEROTIC PLAQUE INSTABILITY AND RUPTURE PREDICTOR

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Background. *Oxidative stress is crucial in developing broad spectrum of diseases, including atherosclerosis and related life-threatening conditions, such as acute coronary syndrome (ACS) mainly caused by atherosclerotic plaque vulnerability.*

Objective. *To clarify the relation between oxidative stress and plaque instability we decided to compare oxidative profiles of patients with acute coronary syndrome (ACS) and with chronic coronary syndrome (CCS), evaluated at admission to the coronary care unit (CCU) of LTD Clinic-LJ (Kutaisi, Georgia) in April 2018 - June 2019, who underwent successful primary percutaneous coronary intervention (PCI).*

Methods. *191 patients were enrolled (100 patients with ACS in Group 1 and 91 patients with CCS in Group 2) into the study. Using the CR3000 FORM PLUS (Callegari Srl, Catellani Group, Italy) – Callegari Point of Care instrument we evaluated free oxygen radical test (FORT), free oxygen radicals defense (FORD), calculated REDOX Index and the overall Profile of oxidative stress.*

Results. *The mean/median concentration of Free Oxygen Radicals was significantly higher in the patients with ACS (404.37±9.83 Fort units/2.36 mmol/l H2O2 eq. vs 282.34±9.83 Fort units/2.36 mmol/l H2O2 eq., p<0.0001). Significant correlation was found between advanced oxidative stress and acute coronary syndrome (OR 14.42 95% CI (7.08-29.4), RR 3.26 95% CI (2.31-4.60) with high diagnostic characteristics (sensitivity of 82% and specificity of 92.3%; positive predictive value of 92% and positive likelihood ratio of 11).*

Conclusion. *Oxidative stress is crucial in life-threatening acute coronary events. Measurement of overall oxidative stress profile, as a surrogate of plaque instability and rupture predictor, could help the clinicians in risk stratification and prevention of acute coronary syndrome (ACS).*

KEY WORDS: **acute coronary syndrome (ACS); chronic coronary syndrome (CCS); oxidative stress; free oxygen radical test (FORT); free oxygen radicals defense test (FORD); REDOX index; oxidative stress profile; vulnerable plaque; plaque rupture.**

Introduction

Coronary heart disease (CHD) has remained the leading cause of death globally in the last two decades. According to the World Health Organization Global Health Observatory (GHO) data IHD is the world's biggest killer, accounting for 9.33 million deaths in 2016 [1].

Acute coronary syndrome (ACS) is a clinical manifestation of CHD with variable consequences range from unstable angina (UA) to non-ST elevation myocardial infarction (NSTEMI), ST-elevation myocardial infarction (STEMI), and sudden cardiac death. Acute coronary syndrome (ACS) mainly as a result of plaque disruption in coronary arteries is responsible for one-third of total deaths in people older than 35 [2].

The main therapeutic approach to ACS is focused on interventional techniques designed to restore blood flow in hemodynamically compromised coronary arteries. However, this reactive strategy has a weak preventive effect on future coronary events. Although pharmacotherapy with antiplatelet agents and statins does not have a dramatic risk-lowering effect as well [3,4].

In the last 10 years, a major improvement has been made in an effort to understand one of the main mechanisms of ACS: the concept of vulnerable plaque as a cause of major ischemic events [5].

The term "vulnerable plaque" was originally used to define a plaque prone to rupture. However, apart from rupture the term "vulnerability" includes other types of lesion, such as plaque erosion, and plaque calcification [6].

The vulnerable plaque is made of a large lipid core (foam cells, apoptotic/necrotic cells, and debris) [7] which is separated from the lumen by a fibrous cap (mainly comprising collagen, proteoglycans, and smooth muscle cells) [8, 9]. Weakening of the fibrous cup under different stressors and a lack of healing results

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in plaque fissuring, thrombus formation and, therefore, in acute coronary syndrome [10, 11].

Many clinical studies support a crucial role for oxidative stress in cardiovascular diseases [12,13]. Oxidative stress (imbalance between enhanced production of reactive oxygen species (ROS) and enzymatic/nonenzymatic antioxidative potential accountable for oxidative modification of low-density lipoprotein cholesterol (LDLc) and cell damage (including endothelial cells of vessels) initiate fatty streak formation, lesion progression, and plaque rupture [14, 15].

It is very important to identify reliable surrogates of plaque instability and predict the highest risk of rupture. The present study intended to determine the oxidative status in patients with acute coronary syndrome (ACS) and to compare with oxidative/antioxidative parameters in patients with stable ischemic heart disease (SIHD). These findings could help assess the risk of stratification and prevent acute coronary syndrome (ACS).

Materials and methods

Study patients

The study sample consisted of 191 patients who were divided into two groups: Group 1 – 100 patients with acute coronary syndrome (ASC) and Group 2 – 91 patients with chronic coronary syndrome (CCS) admitted to the coronary care unit (CCU) of LTD Clinic-LJ (Kutaisi, Georgia) in April 2018 - June 2019, who under_ went successful primary percutaneous coronary intervention (PCI). In the patients with chronic coronary syndrome (CCS) invasive coronary angiography with revascularization was performed in case of high clinical likelihood of obstructive coronary artery disease (OCAD) and severe symptoms refractory to optimal medical treatment, or typical angina at a low level of exercise and clinical prediction of high-risk of events, or left ventricular dysfunction suggestive of CAD.

Patients with a history of coronary revascularization, or with hemodynamically compromised severe myocardial infarction; those recovering cardiopulmonary arrest, decompensated heart failure; and those with valvular heart disease, cardiomyopathy, severe supraventricular/ventricular arrhythmias (including atrial fibrillation) and conductivity disturbances, end-stage renal disease (ESRD), chronic inflammatory conditions, active cancer, type 1 diabetes mellitus (DM) or decompensated type 2 diabetes mellitus (DM); pregnancy; those on hormone replacement therapy (HRT) or oral contraceptive assumption were excluded from the study. No corrections or changes had been made in the ongoing pharmacotherapy of the patients. All essential laboratory tests and FORT/FORD assays were performed during the first hour of admission.

The study was approved by the Ethics Committee (EC) of Tbilisi State Medical University (TSMU) and local EC of LTD Clinic-LJ and a written informed consent was provided by each study participants.

Assay of oxidative stress

The enrolled patients' oxidative status was assessed by measuring ROS damage index (FORT test) and antioxidant capacity (FORD test), and REDOX index calculation using the CR3000 FORM PLUS (CallegariSrl, Catellani Group, Italy) – Callegari Point of Care instrument with following technical characteristics:

Parameters measured and specificity: (i) free oxygen radical test (FORT): overall organic radicals, e.g. Hydroperoxides, (ROOHs)/reactive oxygen species (ROS); (ii) free oxygen radicals defense (FORD): plasmatic antioxidant compounds including vitamin C; proteins (e.g. albumin and ceruloplasmin); bilirubin; thiol groups (e.g. glutathione); polyphenolic compounds (e.g. flavonoids and tannins); (iii) Oxidative-reductive balance (REDOX index): overall score of the oxidation-reduction state. The index was expressed as a number (from 0 to 100) identifying 5 specific profiles (A-E).

Assay principle: (i) free oxygen radical test (FORT): colorimetric method based on the Fenton reaction; (ii) free oxygen radicals defense (FORD): colorimetric method based on the quenching of the color.

Reference range: (i) free oxygen radical test (FORT): Up to 310 Fort units/2.36 mmol/l H_2O_2 eq; (ii) free oxygen radicals defense (FORD): 1.07-1.53 mmol/l trolox eq.;

Sample type: whole blood;

Technique: Point of care analysis via ready to use, wet, disposable reagents;

Wavelength: 505 nm.

The five profiles of oxidative stress were determined by basal FORT and FORD values and REDOX index calculation: (i) Profile A (Ideal/normal values): redox index: 0-25; FORT <300 units/2.36 mmol/l H2O2 eq.; FORD ≥1.08 mmol/l trolox eq; (ii) Profile B (latent oxidative stress): redox index: 25-50; FORT <300 units/2.36 mmol/l H2O2 eq; FORD ≤1.07 mmol/l trolox eq.; (iii) Profile C (compensated oxidative stress): redox index: 50-58.3; 300 < FORT <330 units/2.36

mmol/l H2O2 eq; FORD ≥1.08 mmol/l trolox eq.; (iv) Profile D (at risk of oxidative stress): redox index: 58.3-66.6; 300< FORT <330 units/2.36 mmol/l H2O2 eq; FORD ≤1.07 mmol/l trolox eq.; (v) Profile E (oxidative stress in progress): redox index: 66.6-100; FORT ≥331 units/2.36 mmol/l H2O2 eq; 0.25< FORD <3.00 mmol/l trolox eq.

Statistical analysis

The data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). To identify oxidative status differences between two groups with abnormal distribution nonparametric tests were used: (i) Mann–Whitney U test for 2 samples; (ii) Median Test for K samples to compare medians across the groups, and (iii) Moses extreme reaction for 2 samples to compare ranges across the groups. Odds ratio (OR) and relative risk (RR) assessment was used to quantify the strength of the association between oxidative stress and acute coronary syndrome (ACS). For assessment of sensitivity/specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio, Youden's J statistic, and prevalence cross-tabulation analysis were used. The 95% confidence interval (CI) was used to estimate the precision of the OR. The *p* value of 0.05 was considered significant.

Results

Study population characteristics

Clinical characteristics of the overall study population are shown in Table 1.

There was no statistically significant difference between the study population characteristics, such as age, male gender, BMI, hypertension, dyslipidemia, ongoing smoking, type 2 DM, and medications, such as beta-blockers, calcium channel blockers, ACEIs or ARBs, and statins. Nitrates consumption was much higher in the patients with chronic coronary syndrome (p<0.0001).

Admission oxidative stress parameters

Fig. 1 depicts the level of baseline oxidative stress parameters evaluated in whole blood of Group 1 of the patients with acute coronary syndrome (ACS) and Group 2 of the patients with chronic coronary syndrome (CCS). The concentration of free oxygen radicals (FORT, Fort units/2.36 mmol/l H_2O_2 eq) evaluated in two groups was as follows: in the patients with ACS, 404.37±9.83 Fort units/2.36 mmol/l H_2O_2 eq, and in the patients with CCS, 282.34±9.83 Fort units/2.36 mmol/l H_2O_2 eq (p<0.0001). Free oxygen radicals defense capacity evaluated by the FORD test in the Group 1 and Group 2 were 1.37±0.035 mmol/l Trolox eq. and 1.5±0.045 mmol/l Trolox eq., respectively (p=0.03). The distribution analysis of the calculated REDOX index in the patients with ACS was 69.2±1.47, and in the patients with CCS was 1.5±0.45 (p<0.0001). A nominal equivalent of stress profile in both groups distributed as follows: 4.56±0.1 and 1.92±0.13, respectively in the Group 1 and Group 2 (p<0.0001).

There are reported results of nonparametric analysis of ROS and antioxidative potential across the groups of patients with ACS and CCS (Fig. 2). Th difference between all oxidative parameters was statistically significant (p<0.0001), except free oxygen radicals defense FORD test (p=0.1).

Table 1. Characteristics of study population

	Group 1 (Patients with ACS) n=100	**Group 2 (Patients with CCS) n=91**	***P* value**
Age (years)	51.8±0.78	49.1±1.02	0.236
Male gender, n (%)	74 (74)	52 (57)	0.874
BMI	27.76±0.35	27.81±0.36	0.924
Hypertension, n (%)	51 (51)	41 (45)	0.413
Dyslipidemia, n (%)	62 (62)	56 (61.5)	0.948
Smoking, n (%)	52 (52)	45 (49.5)	0.726
Type 2 DM, n (%)	44 (44)	38 (41.8)	0.755
BB, n (%)	31 (31)	27 (29.7)	0.842
CCB, n (%)	36 (36)	28 (30.8)	0.446
ACEIs/ARBs, n (%)	42 (42)	38 (41.8)	0.973
Statins, n (%)	42 (42)	45 (49.5)	0.238
Nitrates, n (%)	22 (22)	42 (46.2)	<0.0001*

*Notes. * Statistically significant difference; BMI body mass index, BB beta-blockers, CCB calcium channel blockers, ACSEIs angiotensin converting enzyme inhibitors, ARBs angiotensin receptor blockers.*

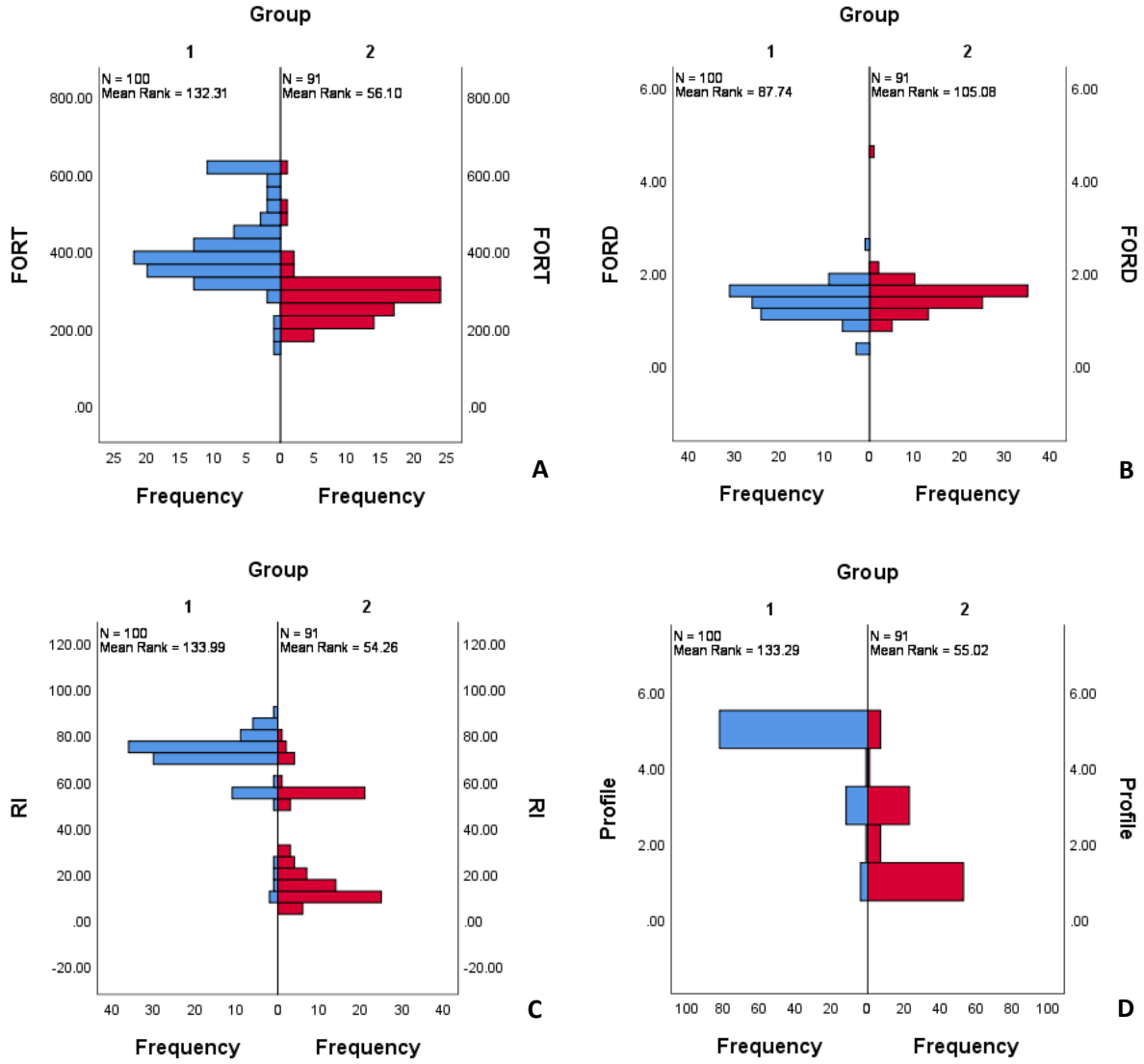


Fig. 1. Distribution of baseline oxidative stress parameters across the groups by the Independent-Samples Mann–Whitney U test.

Notes. Group 1, the patients with acute coronary syndrome (ASC). Group 2, the patients with chronic coronary syndrome (CCS). (A) Distribution of FORT, free oxygen radical test results across the groups (Mann–Whitney U=919, Wilcoxon W=5105, Test statistic=919, Standard error=381.448, Standardized test statistic=-9.519, Asymptotic Sig. (2-sided test) =.000); (B) Distribution of FORD, free oxygen radicals defense test result across the groups (Mann–Whitney U=5376, Wilcoxon W=9562, Test statistic=5376, Standard error=381.480, Standardized test statistic=2.165, Asymptotic Sig. (2-sided test) =.030); (C) Distribution of calculated RI, REDOX index across the groups (Mann-Whitney U=751.5, Wilcoxon W=4937.5, Test statistic=751.5, Standard error=381.173, Standardized test statistic=-9.965, Asymptotic Sig. (2-sided test) =.000); (D) Profile distribution across the groups (Mann–Whitney U=821, Wilcoxon W=5007, Test statistic=821, Standard error=355.1, Standardized test statistic=-10.501, Asymptotic Sig. (2-sided test) =.000).

The evaluation results of association between an exposure (oxidative stress) and an outcome (acute coronary syndrome) are shown in Table 2.

Fig. 3 depicts the stratification of patients in the two groups in line with systemic oxidative stress profile.

A cross-tabulation analysis was performed to assess the sensitivity and specificity, positive and negative predictive values (PPV and NPV, respectively), and positive and negative likelihood ratios (LR+ and LR-, respectively) for systemic oxidative stress in the patients with acute coronary syndrome (Fig. 4). An informedness of sensitivity and specificity data was represented by positive Youden's index (J). There were following diagnostic characteristics of systemic oxidative stress for ACS: sensitivity of 82%, and specificity of 92.3%; positive predictive value (PPV) of 92% versus negative predictive value (NPV) of 82%; positive likelihood ratio (LR+) of 11 versus negative likelihood ratio (LR-) of 0.2.

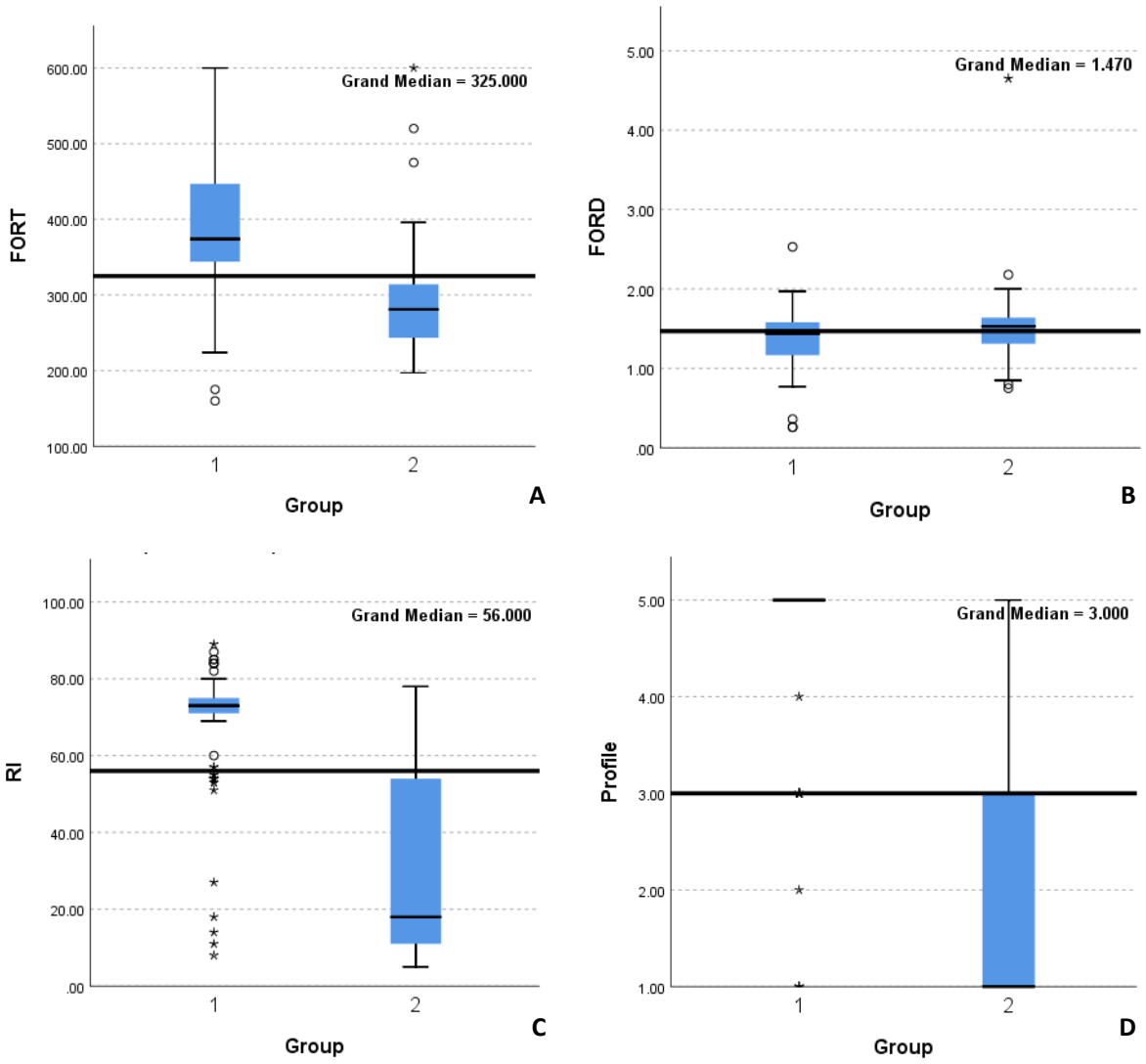


Fig. 2. Independent-Samples Median Test of baseline oxidative stress parameters across the groups.
Notes. Group 1, patients with acute coronary syndrome (ASC). Group 2, patients with chronic coronary syndrome (CCS). (A) FORT, free oxygen radical test results across the groups (Median=325, Test statistic=99.317, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.000; Yates's Continuity correction Chi-Square=96.447, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.000); (B) FORD, free oxygen radicals defense test result across the groups (Median=1.470, Test statistic=3.198, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.074; Yates's Continuity correction Chi-Square=2.7, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.1); (C) RI, REDOX index across the groups (Median=56, Test statistic=104.393, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.000; Yates's Continuity correction Chi-Square=101.454, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.000); (D) Profile across the groups (Median=3.0, Test statistic=105.183, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.000; Yates's Continuity correction Chi-Square=102.229, Degree of freedom=1, Asymptotic Sig. (2-sided test) =.000).

Table 2. The strength of association between oxidative stress and acute coronary syndrome

	Odds ratio (OR)	Relative risk (RR)
	14.42	3.26
Standard Error (SR)	0.36	0.18
Lower 95% confidence Interval (CI)	7.08	2.31
Upper 95% confidence Interval (CI)	29.4	4.60

Discussion

Oxidative stress is one of the key pathogenetic factors for various diseases, including life-threatening conditions, such as acute coronary syndrome (ACS) [16] mainly caused by atherosclerotic plaque instability [14,15].

Available scientific data extend our understanding of the biology of plaque vulnerability

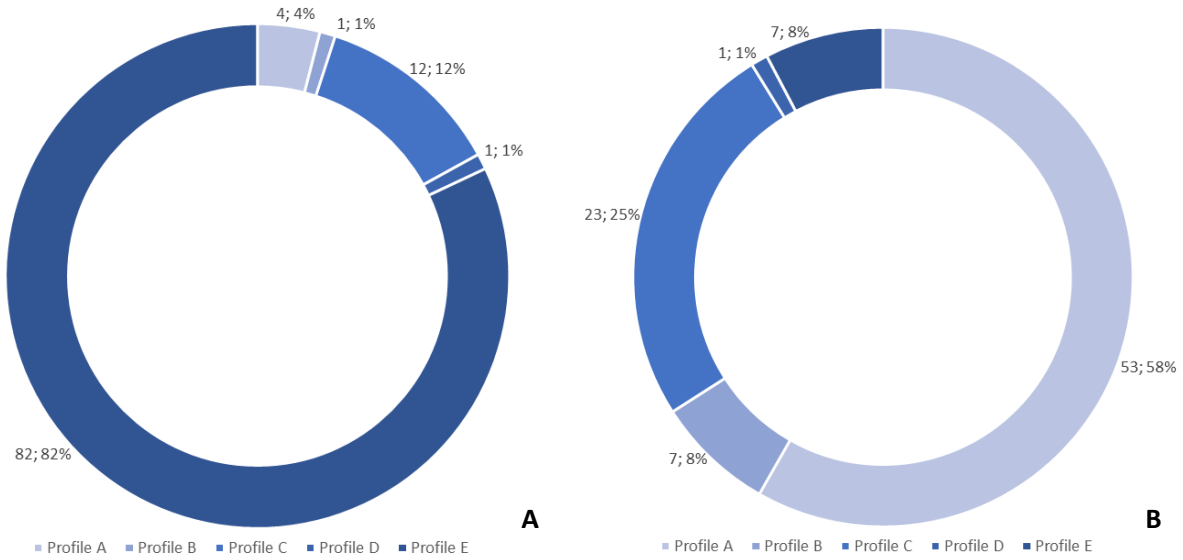
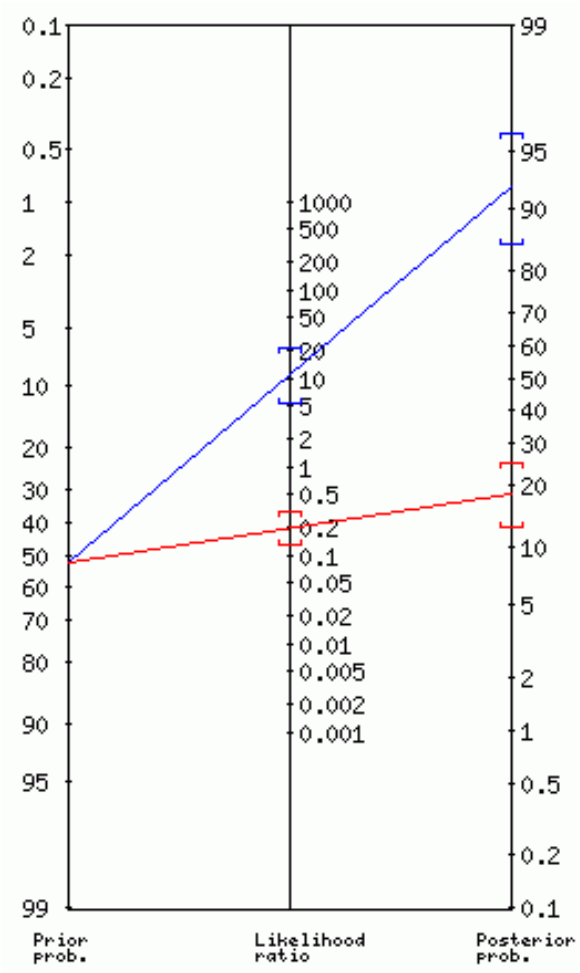


Fig. 3. Doughnut chart of systemic oxidative stress profile frequency in the two groups.
Notes. A. Group 1, patients with acute coronary syndrome (ACS);
B. Group 2, patients with chronic coronary syndrome (CCS);
Profile A, (Ideal/normal values): redox index: 0-25; FORT <300 units/2.36 mmol/l H_2O_2 eq; FORD ≥1.08 mmol/l trolox eq. Profile B, (latent oxidative stress): redox index: 25-50; FORT <300 units/2.36 mmol/l H_2O_2 eq; FORD ≤1.07 mmol/l trolox eq.
Profile C, (compensated oxidative stress): redox index: 50-58.3; 300 < FORT <330 units/2.36 mmol/l H_2O_2 eq; FORD ≥1.08 mmol/l trolox eq. Profile D, (at risk of oxidative stress): redox index: 58.3-66.6; 300< FORT <330 units/2.36 mmol/l H_2O_2 eq; FORD ≤1.07 mmol/l trolox eq. Profile E, (oxidative stress in progress): redox index: 66.6-100; FORT ≥331 units/2.36 mmol/l H_2O_2 eq; 0.25< FORD <3.00 mmol/l trolox eq. p value <.0001.



and suggest the important role of ROS as a plaque stability regulator and mediator of acute coronary events [17]. It appears that ROS production might be considered as a marker of atherosclerotic plaque instability.

The main goal of the present study was to compare the oxidative stress profile between two groups of patients with acute coronary syndrome (ACS) and chronic coronary syndrome (CCS). These findings could help assess risk of stratification and prevent acute coronary syndrome (ACS).

Recent studies have shown the usefulness of the free oxygen radical test (FORT)/free oxygen radicals defense (FORD) novel colorimetric assay for evaluating oxidative stress [18-22].

Fig. 4. The likelihood ratio (LR) nomograms of combination of laboratory and clinical findings in patients with Acute Coronary Syndrome (ACS)
Prior probability (odds): 52% (1.1). Positive test: positive likelihood ratio 11, 95% confidence interval [5.20,22]; Posterior probability (odds) 92% (12.1), 95% confidence interval: [85%,96%]; (~1 in 1.1 with positive test are sick). Negative test: Negative likelihood ratio 0.20, 95% confidence interval [0.13,0.30]; Posterior probability (odds): 18% (0.2), 95% confidence interval [12%,25%] (~ 1 in 1.2 with negative test are well).

Using the CR3000 FORM PLUS (CallegariSrl, Catellani Group, Italy) – Callegari Point of Care instrument we have assessed oxidative stress profile in 191 patients divided into Group 1 and Group 2 with 100 patients with acute coronary syndrome (ACS), and 91 patients with chronic coronary syndrome (CCS), respectively. The characteristics of the study population were well matched between groups (Table 1), except nitrates consumption – it was much higher in the Group 2 in a cohort of patients with chronic coronary syndrome (p<0.0001).

The blood concentration of free oxygen radicals (FORT) at admission in the coronary care unit (CCU) of LTD Clinic-LJ (Kutaisi, Georgia) was much higher in the patients with acute coronary syndrome in comparison of the patients with chronic coronary syndrome: 404.37±9.83 Fort units/2.36 mmol/l H2O2 eq, and 282.34±9.83 Fort units/2.36 mmol/l H2O2 eq., respectively (p<0.0001).

The analysis of an antioxidant defense potential (measured by the FORD test) across the groups have shown higher concentration of free oxygen radicals in cases of acute coronary syndrome (mean value of FORD 1.37±0.035 mmol/l Trolox eq. for ACS versus 1.5±0.045 mmol/l Trolox eq. for CCS).

The calculated REDOX index in the patients with ACS (Group 1) was significantly higher than in the patients with CCS (69.2±1.47 vs 1.5±0.45, p<0.0001).

The analysis of overall oxidative status (derived from FORT/FORD values and calculated REDOX index) across groups has indicated to significant correlation of uncontrolled systemic oxidative stress (Profile E, oxidative stress in progress) with acute coronary syndrome (4.56±0.1 for Group 1 vs 1.92±0.13 for Group 2, p<0.0001). The incidence of Profile E (oxidative stress in progress) in the patients with acute coronary syndrome was 82/100 (82%) versus 7/91 (8%) in the patients with chronic coronary syndrome.

A cross-tabulation analysis has shown high diagnostic characteristics of systemic oxidative stress measurement test in cases of acute coronary syndrome (sensitivity of 82%, and specificity of 92.3%; positive predictive value (PPV); positive likelihood ratio (LR+) of 11).

Finally, we have recognized three main limitations to the presents study. First, this was case-control design study. Second, this was not a multi-center study. Third, the sample size was small. Therefore, we are not able to extrapolate the findings of the present study to the general population.

Conclusions

An extremely strong association between absolute oxidative stress and Acute Coronary Syndrome (OR 14.42 95% CI (7.08-29.4), RR 3.26 95% CI (2.31-4.60), high sensitivity and specificity (82% and 92.3%, respectively), and strong positive prediction and likelihood (positive predictive value of 92% and positive likelihood ratio of 11) indicate to pivotal role of oxidative stress in the development of life-threatening acute coronary events. It seems to be a reliable surrogate of plaque instability and rupture predictor.

The findings of our study could help in risk stratification and prevention of acute coronary syndrome (ACS) in clinical setting.

Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Zaza Lominadze – data curation, formal analysis, funding acquisition, investigation, resources and writing original draft; *Kakhaber Chelidze* – conceptualization, formal analysis, methodology, project administration, supervision, validation and review and editing of original draft; *Levan Chelidze* – formal analysis, software, visualization and review and editing of original draft; *Ekaterine Lominadze* – formal analysis, visualization and review and editing of original draft.

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ОКСИДАТИВНИЙ СТРЕС – ЯК СУРОГАТ НЕСТАБІЛЬНОСТІ КОРОНАРНОЇ АТЕРОСКЛЕРОТИЧНОЇ БЛЯШКИ ТА ПРЕДИКТОР ЇЇ РОЗРИВУ

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Вступ. *Оксидативний стрес відіграє важливу роль у патогенезі багатьох захворювань, в тому числі атеросклерозу та його ускладнень, як гострий коронарний синдром (ГКС), що переважно зумовлений нестабільністю атеросклеротичної бляшки.*

Мета *– з'ясувати взаємозв'язок ступенем оксидативного стресу та нестабільністю бляшки шляхом порівняння оксидативного профілю пацієнтів з ГКС та хронічним коронарним синдромом (ХКС). Дослідження проводили у кардіологічному відділені LTD Клініки-LJ (Кутаїсі, Грузія) в період з квітня 2018 року до червня 2019 року серед пацієнтів, котрим успішно було проведено первинні перкутанні коронарні втручання (ПКВ).*

Методи. *До дослідження було залучено 191 пацієнта (100 пацієнтів з ГКС (Група 1) та 91 пацієнт з ХКС (Група 2). Досліджували такі показники: тест для визначення інтенсивності вільнорадикальних реакцій (Free Oxygen Radical Test, FORT); тест для визначення антиоксидантної здатності (Free Oxygen Radicals Defense Test, FORD); визначали REDOX індекс та Профіль оксидативного стресу за допомогою CR3000 FORM PLUS (Callegari Srl, Catellani Group, Italy).*

Результати. *Концентрація вільних радикалів кисню була достовірно вищою у пацієнтів з ГКС (404,37±9,83 vs 282,34±9,83 Fort од/2.36 ммоль/л H2O2 екв., p<0,0001). Між інтенсивністю оксидативного стресу та розвитком гострого коронарного синдрому встановлено вірогідні кореляційні зв'язки (OR 14,42 95% CI (7.08-29.4), RR 3,26 95% CI (2,31-4,60) з високими діагностичними характеристиками (чутливість 82,0% та специфічність 92,3%; позитивна прогностична роль 92,0% та позитивний коефіцієнт вірогідності 11).*

Висновки. *Оксидативний стрес відіграє вирішальну роль при гострих коронарних подіях, що загрожують життю. Вимірювання оксидативного профілю, як сурогату нестабільності атеросклеротичної бляшки та предиктора її розриву, може допомогти лікарю у стратифікації ризику коронарних подій для попередження гострого коронарного синдрому (ГКС).*

КЛЮЧОВІ СЛОВА: **Гострий коронарний синдром; хронічний коронарний синдром; оксидативний стрес; тест для визначення інтенсивності вільнорадикальних реакцій (Free Oxygen Radical Test, FORT); тест для визначення антиоксидантної здатності (Free Oxygen Radicals Defense Test, FORD); REDOX індекс; профіль оксидативного стресу; нестабільна бляшка; розрив бляшки.**

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NONSPECIFIC DYSPLASIA OF THE CONNECTIVE TISSUE – A FACTOR OF VENOUS THROMBOEMBOLIC COMPLICATIONS IN ENDOPROSTHETICS OF HIP JOINTS

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Background. *Important part of orthopedic surgery is endoprosthetics of hip joints, which eliminates pain syndrome, restores the amplitude of movement and the support ability of lower limbs. But some complications usually take place; venous thromboembolism is leading among them.*

Objective. *The aim of the study was to investigate endothelial dysfunction and activity of the hemocoagulation system at different levels of VTEC risks and thus to work out the strategy of thromboprophylaxis in patients with osteoarthrosis of the hip joint and femoral neck fracture combined with non-specific dysplasia of the connective tissue.*

Methods. *219 patients of a mean age of 64.7±3.8 years old underwent surgery. In 137 (62.1%) cases, a total cement hip replacement was performed for osteoarthritis. 82 (37.4%) patients underwent total and unipolar cement hip replacement for cervical femoral neck fractures.*

Results. *Clinical manifestations of non-specific connective tissue dysplasia were detected in 83 (37.9%) patients that was confirmed by the laboratory determination of the level of general, bound and free oxyproline. In the postoperative period, the thrombotic process in the venous system of the inferior vena cava was diagnosed in 23 (10.5%) cases. The level of indicators of endothelium status dysfunction was much more significant in the patients in cases of nonspecific dysplasia of connective tissue. Operative intervention on the hip joint in the patients with nonspecific dysplasia of connective tissue in 11 (13.3%) cases was complicated by development of venous thrombosis. In the patients without non-specific connective tissue dysplasia, postoperative thrombosis in the system of the inferior vena cava was diagnosed in 12 (8.8%) cases.*

Conclusions. *Patients with osteoarthrosis of the hip joint and the femoral neck fracture accompanied by the non-specific dysplasia of the connective tissue are characterized by high levels of endothelial dysfunction and increased activity of the blood-coagulation system.*

KEYWORDS: **endoprosthetics; thromboembolism; endothelial dysfunction; dysplasia.**

Abbreviations

AT III	antithrombin III
CTD	non-specific connective tissue dysplasia
DVT	deep vein thrombosis
FBA	fibrinolytic blood activity
FDP	fibrin degradation product
FPA	fibrinopeptide A
IVC	inferior vena cava
LMWH	low molecular weight heparins
NCTD	non-specific connective tissue dysplasia
PRT	plasma recalcification time
SCFM	soluble complexes of fibrin-monomers
TFA	total fibrinolytic activity
TPA	thromboembolism of the pulmonary artery
VEGF	vascular endothelial growth factor
IVC	inferior vena cava
VTEC	venous thromboembolic complications

Introduction

Significant occurrence of diseases of the lower extremities joints leads to the search for effective methods of treatment. Among them endoprosthetics of the joints is foremost. It allows eliminating pain syndrome, restoring the amplitude of movement and the support ability of the lower limb [1]. During endoprosthetics, a number of complications often occur; VTEC is leading among them [2]. Without VTE prophylaxis, the overall VTE incidence in medical and general surgery of the hospitalized patients is in the range of 10- 40%, while it ranges up to 40-60% in major orthopaedic surgery. With routine VTE prophylaxis, fatal pulmonary embolism is uncommon in orthopaedic patients and the rates of symptomatic VTE within three months are in the range of 1.3-10% [3]. VTE preventive treatment methods are divided into mechanical and pharmacological. The mechanical include mobilization, graduated compression stockings, intermittent pneumatic compression device and venous foot pumps; the pharmacological include aspirin, unfractionated heparin, low molecular weight heparin (LMWH), adjusted dose vitamin K antagonists, synthetic

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pentasaccharide factor Xa inhibitor and newer oral anticoagulants. LMWH seems to be more efficient overall compared with the other available agents. We are still sceptical about the use of aspirin as a sole method of prophylaxis in total hip and knee replacement and hip fracture surgery, while controversy still exists regarding the use of VTE prophylaxis in knee arthroscopy, lower leg injuries and upper extremity surgery [3]. The factors that predispose a high risk of venous thromboembolism in the patients undergoing endoprosthetics of the joints are taken into account [4]. But there is no significant decrease of frequency of VTEC development [5].

The aim of the study was to investigate endothelial dysfunction and activity of the hemocoagulation system at different levels of VTEC risks and thus to work out the strategy of thromboprophylaxis in patients with osteoarthrosis of the hip joint and femoral neck fracture combined with non-specific dysplasia of the connective tissue.

Methods

219 patients of a mean age of 64.7±3.8 years old underwent surgery. In 137 (62.1%) cases, total cement hip replacement for osteoarthritis was performed. 82 (37.4%) patients underwent total and unipolar cement hip replacement for cervical femoral neck fractures. Operative intervention was carried out under subdural anaesthesia with bupivacaine.

Clinical manifestations of non-specific connective tissue dysplasia (NCTD) were detected in 83 (37.9%) patients. This was confirmed by laboratory determination of the level of general, bound and free oxyproline. The method of P.N. Sharaev for a calibrated curve was used.

Mechanical prophylaxis of VTEC by elastic compression of the lower extremities was used in all cases. Pharmacological prevention of VTEC was performed using LMWH (enoxaparin).

The study included 136 patients (group I) with hip joint osteoarthrosis and femoral neck fracture and 83 patients (group II) with osteoarthrosis of the hip joint and femoral neck fracture and associated NCTD.

In the postoperative period, the thrombotic process in the venous system of the inferior vena cava (IVC) was diagnosed in 23 (10.5%) cases. Operative intervention on the hip joint in the patients with NCTD in 11 (13.3%) cases was complicated by the development of venous thrombosis. In the patients without NCTD, postoperative thrombosis in the venous system of IVC was diagnosed in 12 (8.8%) cases. In 21 patients, postoperative thrombosis was detected in the deep venous system of IVC and only in two cases in the superficial venous system.

Monitoring of the thrombotic process in the venous system of the inferior vena cava was performed by duplex ultrasound scans of the vessels of the lower extremities by Sonoscape S8Exp with a frequency of 5-12 MHz and 2.5-4 MHz from the first day of the postoperative period. In the first day of the postoperative period, in two patients the thrombotic processes in the deep veins of the IVC system was diagnosed, on the 2nd day – in 5 cases, on the 3rd day – in one patient, on the 6th, 7th and 8th days – in 3, 5 and 2 cases, respectively, and on the 11th and 12th day – in one and two patients, respectively. In the superficial venous system of the lower limb, the thrombotic process was detected in two cases on the 6th and 10th day of the postoperative period.

8 (34.9%) cases of the venous thrombotic processes were diagnosed within the first three days of the early postoperative period in the patients with NCTD.

To choose the treatment tactics for venous thrombosis of the IVC system, the determination of thrombogenicity of thrombotic masses was performed. The elastographic properties of the venous thrombus were studied by the ultrasonic system Siemens Acuson S2000 (Germany). At the shear wave expansion speed of 2.5-2.6 m/s there is a high risk of embologenity of the thrombi, at the shear wave expansion speed of 2.7-2.9 m/s there is a moderate risk of embologenity of the thrombi, at the shear wave expansion speed of 3.0 m/s and more there is no threat of embolism.

In two cases on the second day of the postoperative period the propagation of the shear wave at 2.5-2.6 m/s in the thrombotic mass of the vein was established using the ultrasonic system Siemens Acuson S2000 (Germany), which is a significant threat to the development of pulmonary embolism. This was an indication for urgent surgical intervention to eliminate the threat of TPA. In all other cases anticoagulant therapy was performed.

The rate of coagulation and fibrinolytic system functioning was determined: fibrinogen was determined by gravimetric method according to R.A. Rutberg; activity of the fibrin stabilizing factor (F XIII) – by means of the "set for determination of FBA-XIII" of the scientific and production firm SIMKO Ltd (Lviv); thromboplastic activity of blood – by B.A. Kudryashov and

P.D. Ulytina method; Plasma recalcification time – by Bergerhof and Rock method; plasmin, plasminogen, total fibrinolytic activity (TFA) – by V.A. Monastyrska et al. method (1988); time of lysis of euglobin clots – using the "Set for the determination of fibrinolytic blood plasma activity" by the scientific and production firm SIMKO Ltd (Lviv). D-dimer was determined by immunoanalytical method using a coagulometer. The soluble fibrin-monomer complexes were determined using the Tablet method.

The state of the endothelial system was evaluated by determining the level of endothelial dysfunction markers: the concentration of P-selectin, E-selectin, tissue plasminogen activator (t-PA), type 1 vascular endothelium adhesion molecule (sVCA M-1) was determined using the Bender MedSystems (Austria) for immune-enzyme analysis. Endothelin-1 concentration was determined using the Biomedica (Canada) kits for immune-enzyme analysis. The reaction was evaluated on a SUNRISE microplate semimetric photometer (Tecan, Austria) using the Hydroflex washing station (Tecan, Austria). To determine circulating endothelial cells (CEC) the J. Hiadovec and N.N. Pertishchev et al. method (2001) was used. The level of nitrogen oxide (NO) metabolites, vascular endothelial growth factor (VEGF) was determined by the immune enzyme method using the KHGO111-VTGF analyser and the Griess reagent.

Results

The state of the endothelial system reflects the pathology of the organism. In the patients with osteoarthrosis of the hip joint and femoral neck fracture an increased level of all endothelial system markers was noted. At the same time, the level of indicators of endothelium status was more significant in the patients (group II) with pathology of hip joint in the presence of NCDT (Table 1). Thus, at the preoperative stage of treatment, the blood level of circulating endothelial cells was higher in 1.8 times ($p<0.001$), endothelin-1 level was increased in 2.2 times ($p<0.001$), P-selectin and E-selectin levels increased in 1.5 times ($p<0.001$) and 1.2 times ($p<0.05$) respectively, compare to indicators of healthy persons. There was a 1.8 fold ($p<0.001$) increase of blood NO levels and a slight 1.2 fold ($p<0.05$) increase of the VEGF blood contents.

Operative intervention in the patients of group II contributed to development of severe endothelial dysfunction. This was especially noticeable at the twelfth hour of the postoperative period. The latter occurred due to a 1.5 fold increase ($p<0.05$) in the contents of circulating endothelial cells in blood and a 1.5 fold increase ($p<0.05$) in endothelin-1, a 1.2 fold decrease ($p<0.05$) and a 1.5 fold decrease ($p<0.05$) of P-selective and E-selectin levels respectively, to compare with the preoperative period. There was also a slight increase of NO contents in blood and a 1.3 fold ($p<0.05$) increase of VEGF contents in blood.

At the stage of preoperative preparation of patients, activation of the hemostasis system was observed (Table 2). This was more significant among the patients of the group II. The indicators that characterize the condition of the coagulation system of patients with NCTD were in 1.2 times higher than those of the patients without NCTD. In the patients with NCTD in the preoperative period the content of fibrinogen was 5.07±0.35 g/l, (in healthy patients – 3.52+0.34 g/l ($p<0.05$). At the same time, an increase of the contents of SCFM in blood was determined as of 0.57±0.11 extr. units ($p<0.05$). Monomers that were formed as a result of separation of fibrinopeptides A and B from fibrinogen under the influence of thrombin, form macromolecular degradation products of fibrin with it. Trombinemia was confirmed by the increase up to 2.61±0.23 ng/l ($p<0.05$) of FPA content. At the same time, an increased up to 6.92±2.09 μg/l (norm 4.71±1.58 μg/ml) content of the FDP was detected. It took place on the background of minimal changes of the FBA and in the absence of any changes in the level of AT III and PRT.

Significant changes of coagulation system occurred during the intraoperative period of surgical treatment of the patient. At the traumatic stage of surgical intervention, a hypercoagulative condition of blood developed. During that period, the fibrinogen level in the blood exceeded the preoperative level by only 10.2%, then the blood contents of SCFM was increased in 1.4 times ($p<0.05$), the contents of FPA was increased in 1.7 times ($p<0.001$), the contents of FDP was increased in 1.9 times ($p<0.001$). It took place on the background of reduced fibrinolytic activity of blood, reduced contents of AT III in blood, decrease of PRT (Table 3).

The maximum level of hypercoagulation reached at 6 h. of early postoperative period. At that time, the blood fibrinogen level exceeded the preoperative rate in 1.3 times ($p<0.05$), and the contents of SCFM in blood was increased in 2.1 ($p<0.001$) times, the content of FPA and FDP – in 2.7 and 2.8 ($p<0.001$) times, respectively. It took place at a 1.3 fold decrease

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Table 1. Characteristics of the endothelial system in the patients with pathology of the hip joint

Index		CIC, Number of cells ×10⁴/l	sVCAM1, ng/ml	P-selectin, ng/ml	E-selectin, ng/ml	t-PA, ng/ml	Endothelin-1, fmol/ml	NO, μmol/l	VEGF, pk/ml
Group	Normal indices	4.04±1.09	229.43±35.31	154.82±19.54	40.79±10.13	3.68±0.73	4.12±0.35	21.39±4.71	51,43±5,59
Group I	Preoperative	5.56±1. 23*	257.83±26.61	175.10±23.35	49.65±8.17*	3.10±0.30	5.53±0.37	34.16±5.54	57,05±5,35
	Postoperative, 6 hrs.	7.87±2.89**	357.68±29.85**	173.21±17.94	42.27±8.32	2.53±0.38	7.51±0.41	34.29±5.65	59,12±5,76
	Postoperative, 12 hrs.	8.13±3.52**	363.61±31.27**	179.18±18.69	35.69±6.14**	2.25±0.47	6.77±0.45**	37.13±5.13	58,75±8,54
Group II	Preoperative	7.35±1. 51 *	293.57±28.62*	235.16±25.72*	48.43±6.46	2.58±0.35*	9.11±0.45 *	39.21±5.43 *	60,11±6,85
	Postoperative, 6 hrs.	9.15±3.71**	397.14±34.18	199.16±71.59	30.67±7.16**	2.45±0.28	12.58±0.56 **	43.62±5.17	62,27±6,61
	Postoperative, 12 hrs.	10.81±3.61**	415.17±45.23**	194.58±67.27**	32.74±6.36**	2.31±0.25	13.41±0.54**	45.87±5.39	68,21±6,97

*Notes: * – a significant difference between the normal indices and indices before the surgery in the patients of groups I and II.*
*** – a significant difference between the indices of the patients of groups I and II before the surgery and the indices in 6 hrs. and 12 hrs. after the surgery.*

Table 2. The state of hemostasis in the patients with pathology of the hip joint

Index	Preoperative Group I	Preoperative Group II	Normal values
Fibrinogen, g/l	4.16±0.18	5.07±0.35*	3.52±0.34
Soluble complexes of fibrin monomers (SCFM), extr. units	0.48±0.06	0.57±0.11*	0.42±0.04
Fibrinopeptide A, ng/ml	2.04±0.28	2.61±0.23*	1.82±0.24
Fibrin degradation product, mcg/ml	5.26±2.14	6.92±2.09*	4.71±1.58
Fibrinolytic blood activity, %	50.63±0.54	54.49± 0.53	50.63± 0.46
Antithrombin III, %	93.83±7.56	89.77± 8.09	96.72±6.22
Plasma recalcification time, c	104.42±9.68	91.67±10.31	110.15±8.57

*Note: * – a significant difference between the norm and levels in the patients of groups I and II.*

Table 3. The intraoperative state of hemostasis in the patients with hip arthritis and NCTD

Index	Preoperative	Traumatic stage	3hr. p/o	6hr. p/o	12 hr. p/o
Fibrinogen, g/l	5.07±0.35	5.59±0.46	5.82±0.49	6.54±0.48*	6.43±0.47*
SCFM, units.extr.	0.57±0.11	0.81± 0.11*	1.23±0.19**	1.24±0.18**	0.97±0.21**
FPA, ng/ml	2.61±0.23	4.51±0.36*	7.05±0.35*	6.94±0.43*	6.39±0.48*
FDP, mcg/ml	6.92±2.09	13.47±3.56*	18.25±4.41*	19.12±4.38*	14.89±4.78*
FBA, %	54.49±3.53	43.75±4.13	42.19±4.16*	43.41±4.23*	45.72±4.27*
AT III, %	89.77+6.09	84.12+6.55	81.24+5.49	84.54+5.75	85.56+5.68
PRT, sec	91.67±7.31	80.57±7.33	82.65±7.72	84.59±7.37	89.28±8.19

*Notes: * – p<0.05 in comparison with preoperative indices;*
*** – p<0.001 in comparison with preoperative indices.*

(p<0.05) of fibrinolytic activity of blood, a decrease of the contents of AT III, and a slight decrease of PRT.

Operative intervention significantly influenced the aggregation state of patients' blood. So, at the traumatic stage of surgical intervention and up to 6 hours of the early postoperative period, the platelet aggregation rate significantly increased with a slight decrease in platelet aggregation time. In the same period, there was a decrease in the activity of the fibrinolytic blood system. This level of anticoagulant system with a tendency to exacerbation was maintained until the 3rd h. of early postoperative period. From the 6th hour of early postoperative period a gradual increase in the activity of the fibrinolytic blood system was evidenced.

The results of the study of hemostasis in the patients with NCTD at the intraoperative stage of surgical intervention at the increase of hypercoagulative properties of blood, strengthening its aggregation ability with a depressed state of the fibrinolytic blood system indicated development of conditions for thrombotic process formation in the venous system. It could be promoted by a high level of endothelial dysfunction, which increased in the conditions of surgical intervention.

Discussion

A number of complications often occurs in cases of endoproathetics. VTEC among them is still urgent [1]. Patients with surgical interventions on the large joints of the lower extremities require anticoagulant prophylaxis [2]. Both the classical factors of the Virchow's triad, and the specific factors, which are specific for the endoprosthetics of the hip joint [5], as well as individual factors of the patients risk and pathology of large joints are taken into account [5]. However, in thromboprophylaxis the incidence of thrombosis of deep veins after the hip replacement is 0.8-9.0%, pulmonary embolism – from 1.4 to 6.0% [6]. The latter gives the right to suppose that during the thromboprophylaxis the factors with different etiopathogenetic origin are not taken into account, but there is a direct or indirect activation of procoagulant FBAs in their presence. Thus, much attention should be paid to nonspecific dysplasia of the connective tissue [7].

The patients with hip osteoarthrosis, femoral neck fracture with NCTD are characterized by increased degree of endothelial dysfunction, a greater activity of the haemocoagulation system compared to the patients without NCTD. Thus, in the preoperative stage, the blood level of markers of endothelial dysfunction in the patients with NCTD is in 1.5-2.2 times (P <0.001) higher compare to the patients without NCTD. It causes a 1.2 fold higher activity of the blood hemocoagulation system of these patients.

The patients with pathology of the hip joint with NCTD experience an increased degree of endothelial dysfunction at the intraoperative stage which leads to hypercoagulation. These changes of the endothelial system and the hemocoagulation system create favourable conditions for VTEC.

Endoprosthetics of the hip joint is a high-risk group of VTEC and requires anticoagulation prophylaxis. A number of present recommendations offer pharmacological prophylaxis for prevention of VTEC in cases of endoprosthetics of large joints. The use of low molecular heparins, synthetic inhibitors of Xa and IIa factors of blood coagulation or antagonists of vitamin K is recommended for this [8]. However, the rate of VTEC development in the postoperative period is trill at the previous level.

In order to reduce the postoperative DVT, attention should be paid to intraoperative changes in the haemocoagulation state of the operated patients. [9] There are reports [10]

about the diagnosis of the thrombotic process in the IVC system after the end of the surgical intervention.

During the surgical intervention for the pathology of the hip joint from the second half of the traumatic phase and during the first 2-3 hours of the early postoperative period, an increase of the level of hypercoagulation due to fibrin-monomeric complexes is observed. It is the unfractionated heparin that has a predominant influence on the IIa factor (thrombin – fibrinogen) of the hemocoagulation cascade. And thromboprophylaxis should be started straight after the end of the surgical intervention with the prescription of non-fractional heparin and should be continued until the first injection of LMWH, which has a predominant influence on the Xa factor of the blood coagulation system [11].

The use of oral form of unfractionated heparin is promising [12]. This has become possible due to the combination of unfractionated heparin and the molecule N [8 (2-hydroxybenzene) amine] carrier – sodium caprylate. The third phase of coagulation cascade study showed that oral heparin reduces the incidence of postoperative thrombotic formation.

Conclusions

The patients with osteoarthrosis of the hip joint and femoral neck fracture and non-specific dysplasia of the connective tissue are characterized by expressed levels of endothelial dysfunction and increased activity of the hemocoagulation system.

In the postoperative period after endoprosthetics of hip joints, in the patients with osteoarthrosis of the hip joint and femoral neck fracture and non-specific dysplasia of the connective tissue, VTEC was diagnosed in 13.3% of cases, in the patients with osteoarthrosis of the hip joint and femoral neck fracture in the absence of nonspecific dysplasia of the connective tissue VTEC was revealed in 8.8% of cases.

The thromboprophylaxis of VTEC in the patients with endoprosthetics of hip joints should be started straight after the start of surgical intervention with the prescription of non-fractional heparin, which has a predominant effect on the IIa FBA of the hemocoagulation cascade and should be continued until the first injection of LMWH, which has a dominant influence on Ha factor of the hemocoagulation system.

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Conflict of Interests

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НЕСПЕЦИФІЧНА ДИСПЛАЗІЯ СПОЛУЧНОЇ ТКАНИНИ – ФАКТОР РИЗИКУ ВЕНОЗНИХ ТРОМБОЕМБОЛІЙ ПРИ ЕНДОПРОТЕЗУВАННІ КУЛЬШОВОГО СУГЛОБА

І.К. Венгер, Н.І. Герасимюк, С.Я. Костів, І.І. Лойко, Д.В. Хвалибога

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

Вступ. *Важливою складовою ортопедичної хірургії є ендопротезування тазостегнових суглобів, що усуває больовий синдром, відновлює амплітуду рухів та підтримуючу здатність нижньої кінцівки. Але ці втручання супроводжуються рядом ускладнень, серед яких провідне місце займає венозна тромбоемболія.*

Мета. *Дослідити ендотеліальну дисфункцію та активність системи гемокоагуляції на різних рівнях ризиків венозного тромбемболізму та, таким чином, розробити стратегію тромбопрофілактики у пацієнтів з остеоартрозом з переломом шийки тазостегнового суглоба та стегнової кістки у поєднанні з неспецифічною дисплазією сполучної тканини.*

Методи. *Оперовано 219 пацієнтів із середнім віком 64,7±3,8. У 137 (62,1%) спостереженнях проведено тотальне цементне ендопротезування стегна з остеоартритом. 82 (37,4%) пацієнтів отримали тотальну та однополярну заміну стегна при переломах шийки стегнової кістки.*

Результати. *Клінічні прояви неспецифічної дисплазії сполучної тканини були виявлені у 83 (37,9%) пацієнтів, що було підтверджено лабораторним визначенням рівня загального, зв'язаного та вільного оксипроліну. У післяопераційному періоді в 23 (10,5%) спостереженнях діагностували тромботичний процес у венозній системі нижньої порожнистої вени. Рівень показників ендотеліальної дисфункції був суттєво вираженіший у пацієнтів за наявності неспецифічної дисплазії сполучної тканини. Оперативне втручання на тазостегновому суглобі у пацієнтів з неспецифічною дисплазією сполучної тканини у 11 (13,3%) випадках ускладнилось розвитком венозного тромбозу. У пацієнтів без неспецифічної дисплазії сполучної тканини післяопераційний тромбоз у системі нижньої порожнистої вени був діагностований у 12 (8,8%) спостереженнях.*

Висновки. *Пацієнти з остеоартрозом тазостегнового суглоба та переломом шийки стегнової кістки, що супроводжуються неспецифічною дисплазією сполучної тканини, характеризуються вираженим рівнем ендотеліальної дисфункції та підвищеною активністю системи згортання крові.*

КЛЮЧОВІ СЛОВА: **ендопротезування; тромбемболія; ендотеліальна дисфункція; дисплазія.**

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NECROTIZING PANCREATITIS: THE WAYS OF IMPROVEMENT OF SURGICAL TREATMENT

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Background. *In connection with a steady increase of patients with destructive forms of acute pancreatitis, the proportion of which takes from 10-15 to 20-30%, despite of the wide range of treatments for non-biliary necrotizing pancreatitis, the rate of mortality reaches 80-90% in case of infected forms and needs further improvement of therapies.*

Objective. *The purpose of this study is to improve the results of treatment of patients with nonbiliary necrotizing pancreatitis, by optimizing surgical tactics.*

Methods. *The study examined the cases of 120 patients, whose age ranged from 22 to 83 years, including patients under the age of 50 years old who accounted for 60.0%. There were 80 males (66.7%) and 40 females (33.3%). Disease duration up to 24 hours was found in 36 (30.0%) patients, from 25 to 72 hours - in 25 (20.8%) individuals, more than 72 hours - in 49 (49.2%) patients.*

Results. *The research claims in non-necrotizing pancreatitis, left-sided retroperitoneal phlegmon often develops in 64.2% (mortality rate 26.0%) of patients, right-sided in 24.2% (mortality rate 6.8%) of patients, and bilateral - in 11, 6% (mortality rate 64.2%) individuals. Moreover, the highest mortality was observed with a combination of retroperitoneal phlegmon cellulose and abscess of the pancreas and/or omental bursa - 39.1%.*

Conclusions. *The use of various surgical interventions depending on the involvement in the pathological process of various sections of the abdominal cavity/ retroperitoneal space, which made it possible to reduce mortality and hospitalization time of patients in the hospital by 1.5-2 times is proposed.*

KEY WORDS: **necrotizing pancreatitis; lumbotomy; parapancreatic fiber; phlegmon of the retroperitoneal tissue; laparotomy; omentobursostomy.**

Introduction

During the last decade, a steady increase in patients with destructive forms of acute pancreatitis has been observed, the proportion of which takes from 10-15 to 20-30% at the pathology of the abdominal organs. According to many authors, the main causes of high mortality rate are the duration of the disease, the prevalence of pancreatic necrosis and parapancreatic fiber, inadequate selection of the method of surgical and anesthesia, and drug therapy [1, 2, 3]. The variety of surgical treatment (from traditional laparotomy, lumbotomy to puncture-draining operations under the ultrasound control, using various methods of drainage of the abdominal cavity and retroperitoneal tissue) is determined by the clinical form of infected pancreatitis (abscess of the pancreas and/or the lesser sac, the abscess of the abdominal pancreatitis and/or the lesser sac, the abscess of the abdominal pancreatitis and/or the omental purulent peritonitis). Extraperitoneal access to infected foci of pancreatic necrosis is one of the options for surgical treatment of necrotizing pancreatitis, the advantages of which are less trauma, the absence of infection of the abdominal cavity, a reduction in the number of complications (intestinal fistula, bleeding), the possibility of epidural anesthesia, faster recovery of intestinal function and early enteral nutrition [4, 5, 6].

The peak of surgical activity occurs during 2-3 weeks of illness when necrosis of the pancreas and parapancreatic fiber has already developed certain limits (the prevalence of pancreatic necrosis), the process of biological sequestration is completed and the possibility of removal of sequestered and necrotized tissues has appeared. During the primary operation, it is not always possible to visualize the boundaries of healthy and necrotic modified pancreatic tissue and parapancreatic fiber which limits necral and sequestrectomy due to possible damage to the parenchyma of the

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gland and the threat of bleeding that makes it impossible for one-time sanitation and adequate drainage of all purulent-necrotic cavities. Therefore, a number of authors propose to leave non-invested tissues, to drain the affected section of the retroperitoneal tissue, suggesting further programmatic step-by-step revisions and sequestrectomy. The clinical meaning of the division of sanations into stages is also due to the non-simultaneous maturation of sequesters, the secondary progression of the purulent process according to the type of leakages, the danger of septic shock with the simultaneous opening of extensive retroperitoneal ulcers [7, 8, 9].

Despite the wide range of treatments for infected necrotizing pancreatitis, the rate of mortality remains quite high. When analyzing the causes of deaths, inadequate drainage of the pancreatic area with necrosis of parapancreatic fiber, the formation of phlegmon or multiple abscesses in the retroperitoneal space is almost always established [10, 11, 12].

The **objectives** of this study are to improve the results of treatment of patients with non-biliary necrotizing pancreatitis, by optimizing surgical tactics (use of lumbotomy), depending on the prevalence of necrosis in the pancreas and retroperitoneal tissue.

Methods

The study examined the cases of 120 patients who were hospitalized at the Mariupol City Pancreatic Center at the Department of Surgery, Endoscopy and Reconstructive Surgery at Donetsk National Medical University from 2014-2018. The age of patients ranged from 22 to 83 years, including patients under the age of 50 years old who accounted for 60.0%. There were 80 males (66.7%) and 40 females (33.3%). Disease duration up to 24 hours was found in 36 (30.0%) patients, from 25 to 72 hours – in 25 (20.8%) individuals, more than 72 hours – in 49 (49.2%) patients.

The study was conducted in accordance with the principles of bioethics, patients had given informed consent.

The causes of acute non-biliary necrotizing pancreatitis were the following ones: unilateral feeding (excessive intake of predominantly fatty foods) – in 62 (51.7%) patients and intake of alcohol (alcoholic excess) or its surrogates – in 58 (48.3%) individuals.

Limited pancreatic necrosis (less than 30% necrosis of pancreatic parenchyma) was found in 10 (8.3%) patients, the spread one (from 30 to 50% necrosis of pancreatic parenchyma) – in 68 (56.7%) individuals, and subtotal-total (more than 50% of necrosis of the pancreatic parenchyma) – in 42 (35.0%) patients.

Retroperitoneal cellulose phlegmon with diffuse purulent peritonitis due to involvement in the inflammatory process of the peritoneum was detected in 9 (7.5%) patients, retroperitoneal cellulitis without peritonitis – in 90 (75.0%) individuals, and a combination of retroperitoneal phlegmon and pancreatic abscess and/or omental patient bag – in 21 (17.5%) cases.

Left-side retroperitoneal phlegmon was detected in 77 (64.2%) patients, right-side retroperitoneal phlegmon in 29 (24.2%), and bilateral retroperitoneal phlegmon – in 14 (11.6%). Verification of phlegmon of the retroperitoneal tissue was carried out on the basis of data obtained during the ultrasound examination, computer tomography, and surgery (macroscopic evaluation of retroperitoneal tissue – sequestration, detritus), as well as bacteriologically.

All patients received intensive therapy, including hunger, gastric drainage, catheterization of the central vein and bladder, infusion-transfusion therapy in a volume of not less than 40 ml / kg body weight with a ratio of colloidal and crystalloid solutions of 1:4; analgesia: epidural blockade at the level of Th7-9 (method of choice: continuous infusion of 0.2% lidocaine solution with a speed of 6-12 ml/hour), non-steroidal anti-inflammatory drugs (ketoprofen 100 mg 3 times a day); antisecretory therapy (the optimal period is the first three days of the disease): the drugs of choice are sandostatin (octreotide) 100 mg 3 times a day subcutaneously and omeprazole 40 mg 2 times a day IV; reserve drugs 5 – fluorouracil (5% – 5 ml i.v.) and quamel 40 mg 2 times a day i.v. antienzyme therapy (optimal duration – the first 5 days of the disease): contraction of at least 50,000 units/day, pride at least 500,000 units/day; prevention of purulent complications: pefloxacin, 400 mg 2 times a day, i.v. + metronidazole, 500 mg, 3 times a day, i.v., with signs of secondary infection: cefoperazone / sulbactam, cefepime, imipenem, meropenem. With the ineffectiveness of the above activities, resorted to extracorporeal detoxification methods (lympho-sorption, plasmapheresis, etc.). However, the effect was temporary and unstable, which required the need for rehabilitation of extensive purulent-necrotic foci with laparotomy followed by closed drainage of the abdominal cavity and retroperitoneal space.

Results

A total of 31 (25.8%) patients died after surgery. The causes of death in 29 patients were sepsis and multiple organ failure, in 1 case – sepsis and multiple intestinal fistulas, in 1 case – erosive bleeding from the splenic artery.

The severity of necrotizing pancreatitis during the first 24 hours was assessed according to the criteria of systemic inflammatory response syndrome (2 or more signs) and additional risk factors: patient age, body mass index (over 30), hematocrit, APACHE II score (more than 8 points), C-reactive protein indicator (more than 120 mg/L). And after 24 hours – on the scales of assessment of Marshall's multi-organ dysfunction (MODS) or SOFA.

Lumbotomy was performed under general anesthesia or epidural anesthesia. The lumbotomy of the left mini-access was performed as follows: below the arc of the XII rib, the skin and subcutaneous tissue is dissected 2-3 cm from the middle axillary to the back axillary line, pushing the muscle mass apart, the parietal peritoneum is exposed. Paracolous fiber is reached with the fingers, exfoliating the peritoneum medially, focusing on the lower pole of the spleen and the left kidney. The abscess was opened by means of an electric suction, the pus was removed, the retroperitoneal fiber affected by enzymatic aggression (free-lying sequesters). Necrectomy is not performed due to the risk of damage to the "healthy" parenchyma of the pancreas, parapancreatic fiber and large vessels. The purulent necrotic cavity was sanitized and drained by elastic silicone drains. The lumbotomy of the right mini-approach is similar to the method described above, but the guidelines for advancing in the retroperitoneal space are the lower edge of the right lobe of the liver (its posterior surface), the right kidney, the head of the pancreas, the hepatoduodenal ligament.

When the purulent-necrotic lesion spreads to the paracolous tissue, the dissection and drainage of the abscesses was performed through the front mini-accesses in the left or right iliac regions, respectively, using the Volkovich incision up to 4-5 cm long. The iliac regions were moved apart. The parietal peritoneum with the mesentery of the sigmoid (cecum) intestine inwardly exfoliated by stupid fingers and wet tupffers. Next, the surgeon with his fingers penetrates into the paracollate tissue of the retroperitoneal regions. Tissue separation should occur easily and almost bloodless. Flow-through drainage of phlegmon on the left was performed by two approaches: lumbotomy with a mini-access to the left and anterior mini-access in the iliac region. Separation of retroperitoneal tissue from the anterior approach on the right should be made to the level of the pancreatic head.

The purpose of surgical intervention (lumbotomy) in case of infected parapancreatitis is adequate opening and drainage (by elastic silicone drains) of the abscess with destruction of cell bridges, leakages, removal of purulent exudate, loosely sequestered, creating a single well-drained cavity for the next year. In an emergency procedure, about retroperitoneal phlegmon and diffuse purulent peritonitis, caused by the involvement of the parietal peritoneum in the inflammatory process, 9 (7.5%) patients were operated on. All of them performed laparotomy, dissection of phlegmon, sanitation and drainage of the stuffing box and abdominal cavity. In 6 patients, the operation was completed by imposing a laparostomy and in the postoperative period, programmed rehabilitation of the abdominal cavity was performed, and in 1 patient the left lumbotomy was also performed. 3 patients died with left-sided localization of the abscess; postoperative mortality was 22.2%. The cause of death was sepsis and multiple organ failure.

Discussions

Retroperitoneal cellulose phlegmon without peritonitis was an indication for performing surgical interventions in 88 (73.3%) patients. 20 patients died; the mortality rate was 22.7%. The cause of death was sepsis and multiple organ failure.

In 40 (45.4%) patients, laparotomy, dissection and drainage of retroperitoneal phlegmon, sanitation and drainage of the abdominal cavity were used as a surgical aid. In 3 (7.9%) patients, surgery was supplemented with lumbotomy. 14 patients died, including 8 patients with left-sided retroperitoneal cellulitis, 2 individuals with right-sided and 4 patients with bilateral ones. Mortality rate was 32.5%. The cause of death was sepsis and multiple organ failure.

In 24 (27.3%) patients, puncture-draining surgical interventions under the control of ultrasound (POD-UZ) were applied. In addition, 9 patients underwent lumbotomy and 1 – laparotomy, sequestrectomy, omentobursostomy due to the localization of sequesters in the pancreatic head region. 4 (16.6%) patients died, sepsis and PON were the causes of death, and in 1 patient was with bleeding from acute gastric and duodenal ulcers.

In 24 (27.3%) patients with phlegmon of the retroperitoneal tissue in the form of "purulent cells", lumbotomy, sequestrectomy, and drainage of the retroperitoneal tissue were performed. 3 patients underwent laparotomy, sequestrectomy, omentobursostomy in connection with the localization of sequestrum in the pancreatic head region; 2 patients died. The rate of mortality was 8.3%. In 1 case, there was a left-sided lesion of retroperitoneal fat, in 1 patient – bilateral one.

Approximately 23 (19.2%) patients were operated on the combination of retroperitoneal phlegmon with abscess of the pancreas and/or omental bursa. After surgery, 9 patients died, the mortality rate was 39.1%. In 8 (30.4%) patients, laparotomy, necropsy and drainage of abscesses, sanitation and drainage of the abdominal cavity were used as surgical aids. 5 patients died, mortality was 62.5%. All the dead individuals had a left-sided lesion of retroperitoneal fat. The cause of death in 4 patients was sepsis and multiple organ failure, in 1 – sepsis and multiple intestinal fistulas.

A combination of various surgical procedures was used in 15 (65.2%) patients with retroperitoneal phlegmon cellulose. In 13 patients, a pancreas abscess and/or omental bursa was performed with PDO-UZ, and about retroperitoneal phlegmon – lumbotomy, sequestrectomy, drainage of retroperitoneal fat. Including 2 patients, a laparotomy and an omentobursostomy were performed by the third stage in order to remove large sequesters located in the region of the head and body of the pancreas.

In 2 patients at the first stage, PDO-UZ about the retroperitoneal phlegmon in the form of fluid accumulation were performed, followed by laparotomy, omentobursostomy, opening of the stuffing box abscess, sequestrectomy. In total, 4 patients died in this subgroup, the mortality rate was 26.7%. In 2 patients, left-sided localization of retroperitoneal phlegmon cellulose was detected, in 2 patients, bilateral. The causes of death were sepsis and multiple organ failure – in 3 patients, bleeding from the arrosive splenic artery – in 1 patient.

The analysis of the data suggests that left-sided retroperitoneal phlegmon often develops in 64.4% of patients with infected non-biliary necrotizing pancreatitis. Right-sided retroperitoneal phlegmon was found in 25% of patients, and bilateral – in 10.6%. At the same time, the highest mortality rate is observed with bilateral phlegmon of retroperitoneal tissue – 63.6%, with the left-sided it was – 26.9%, and with the right-sided – 7.7%. Moreover, the highest mortality is observed with a combination of retroperitoneal phlegmon cellulose and abscess of the pancreas and/or omental bursa – 41.2%. With phlegmon of the retroperitoneal fiber and diffuse purulent peritonitis caused by the involvement of the parietal peritoneum in the inflammatory process, it was 28.6%, and with the abscess of the retroperitoneal fat without peritonitis – 22.5%.

The analysis of the results of the use of various surgical interventions in the case of phlegmon of the retroperitoneal tissue suggests that the use of laparotomy in the case of the phlegmon of the retroperitoneal tissue is accompanied by the highest mortality rate – 34.9%. In this regard, this operation should not be used to treat retroperitoneal phlegmon with the exception of patients with retroperitoneal cellulitis and diffuse purulent peritonitis, due to the involvement of the parietal peritoneum in the inflammatory process. Somewhat better results were obtained from the use of puncture – draining operations under the control of ultrasound – 16.7%. However, according to our data, they were effective in 28.6% of patients with retroperitoneal phlegmon cellulose in the form of free fluid accumulation. Mortality after lumbotomy was 13.3%. This operation was performed in 71.4% of patients with retroperitoneal phlegmon cellulose in the form of "purulent cells", incl. in 21.4% of patients in whom the use of PDO-Ultrasound was ineffective. Thus, the opening of the retroperitoneal tissue from the mini-accesses (lumbotomy, sequestrectomy, drainage of the retroperitoneal tissue) is the most popular surgery for retroperitoneal phlegmon. However, as our experience has shown one opening of retroperitoneal phlegmon cellulose is not enough. In the absolute majority of patients, there was a need to perform step-by-step rehabilitation of retroperitoneal fat (step-down sequestrectomy).

Comparing the results of our patients' treatment with similar indicators of other medical institutions, similar methods of surgical interventions were used, we established certain features (Table 1).

In the most severe category of patients, where diffuse purulent peritonitis and retroperitoneal phlegmon were combined, the rates of postoperative mortality did not differ significantly among themselves, they also did not have significant differences in patients with isolated phlegmon of retroperitoneal tissue without peritonitis.

Table 1. Comparative results of treatment of patients with the traditional method and with the use of puncture-draining operations

Patient categories	Indicators of treatment					
	Repeated surgical interventions (progressive diseases)		Postoperative mortality		Average length of hospital stay	
	Types of surgical interventions					
	Puncture-draining operations and/or lumbotomy with mini-access	Traditional interventions laparotomy and/or lumbotomy	Puncture-draining operations and/or lumbotomy with mini-access	Traditional interventions laparotomy and/or lumbotomy	Puncture-draining operations and/or lumbotomy with mini-access	Traditional interventions laparotomy and/or lumbotomy
Isolated abscess and/or phlegmon-retroperitoneal tissue (purulent-necrotic phase)	3,3%	6,5%	39,1%	48,2-62,5%	32,5±2,5	48±3,6
Phlegmon-retroperitoneal tissue in the form of an accumulation of free fluid and/or an abscess of the pancreas of the omental bursa	2,8%	5,4%	26,7%	36,5-42,2%	28,7±2,5	46±2,8

But when using puncture-draining operations of the pancreas abscesses and opening the phlegmon of retroperitoneal tissue from a mini-access of our patients, it made it possible to reduce slightly the mortality rates in comparison with other clinical hospitals. This figure of our patients was 39.1% as in other clinics reached 62.5-48.2% [1, 3]. Also, significant differences in the results of treatment of our patients were obtained with the early use of puncture-draining operations for fluid accumulation in the retroperitoneal tissue and isolated abscess of the pancreas and/or omental bursa – 27.6% in our clinic and 36,6-42.2 in other medical institutions [11, 6].

At the same time, it is recommended to use PDO-Ultrasound for abscesses of the pancreas and/or omental bursa, and laparotomy, sequestrectomy and otobursostomy to remove sufficiently large sequesters located in the head and body of the pancreas.

Conclusions

In cases of retroperitoneal fat phlegmon and diffuse purulent peritonitis caused by involvement of parietal peritoneum in the inflammatory process, the preferred treatment is laparotomy, sanitation, and drainage of abdominal, extraperitoneal phlegmon dissection, and retroperitoneal fiber drainage draining followed by with the subsequent programmed sanitation of the abdominal cavity and retroperitoneal tissue.

In cases of postnecrotic infected pancreatic and/or parapancreatic fluid accumulations in the retroperitoneal tissue (abscess of the retroperitoneal tissue) in the form of purulent soaking of cellulose spaces of the “honeycomb” type, the operation of choice is the direct surgical intervention on the pancreas and retroperitoneal tissue from the mini-access (extraperitoneal access - lumbotomy), followed by staged sequestrectomy.

Puncture-draining surgical interventions under the ultrasound control can only be used with retroperitoneal phlegmon cellulose in the form of free fluid accumulation.

In the presence of sequesters of large size, which are located in the head and body of the pancreas, the operation of choice is a laparotomy, sequestrectomy, omentobursostomy.

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НЕКРОТИЧНИЙ ПАНКРЕАТИТ: ШЛЯХИ УДОСКОНАЛЕННЯ ХІРУГІЧНОГО ЛІКУВАННЯ

О.В. Розенко
ДОНЕЦЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ, УКРАЇНА

Вступ. *У зв'язку з неухильним зростанням кількості пацієнтів із деструктивними формами гострого панкреатиту, питома вага якого складає від 10-15 до 20-30%, незважаючи на широкий спектр способів лікування небіліарного некротичного панкреатиту, летальність досягає у випадку інфікованих форм 80-90% і потребує подальшого дослідження та поліпшення методів лікування.*

Мета. *Метою дослідження є поліпшення результатів лікування хворих на небіліарний некротичний панкреатит шляхом оптимізації хірургічної тактики.*

Методи. *До дослідження залучено 120 пацієнтів віком від 22 до 83 років, причому пацієнти віком до 50 років склали 60,0%. Чоловіків було 80 (66,7%), жінок – 40 (33,3%). Тривалість захворювання до 24 годин визначено у 36 (30,0%) хворих, від 25 до 72 годин – у 25 (20,8%), більше ніж 72 години – у 49 (49,2%).*

Результати. *Установлено, що у випадку небіліарного некротичного панкреатиту частіше розвивається лівобічна заочеревинна флегмона – у 64,2% (летальність 26,0%) пацієнтів, правобічна виявлена у 24,2% (летальність 6,8%) пацієнтів, а двобічна – у 11, 6% (летальність 64,2%). Також найбільш високу летальність засвідчено в поєднанні флегмони заочеревинної клітковини й абсцесу підшлункової залози і/або сальникової сумки – 39,1%.*

Висновки. *Автори дослідження запропонували різні варіанти хірургічного втручання залежно від залучення до патологічного процесу різних відділів черевної порожнини/заочеревинного простору, що дозволило знизити летальність і час перебування пацієнтів у стаціонарі в 1,5-2 рази.*

КЛЮЧОВІ СЛОВА: **небіліарний панкреатит; люмботомія; парапанкреальна клітковина; флегмона заочеревинної клітковини; лапаратомія; оментобурсостомія.**

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CHROMOSOMAL DISEASES IN THE HUMAN PATHOLOGY (review)

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Background. *Chromosomal diseases are the cause of 45-50 % of multiple birth defects. Basic research on mutations is performed using genomic technologies to identify a correlation between genotype and phenotype in aneuploidies and to understand its pathogenesis.*

Objective. *The aim of the research is to study the etiology, pathogenesis of symptoms and diagnostics for patients with Down, Klinefelter, Turner syndromes and double aneuploidies by 21 and sex chromosomes.*

Methods. *A literature review by the keywords "Down syndrome", "Klinefelter syndrome", "Turner syndrome", "double aneuploidy" for the period of 2000-2020 was carried out.*

Results. *Down, Klinefelter and Turner syndromes are the most common aneuploidy among viable newborns. Frequency of meiotic non-disjunction events causing these aneuploidies increases with the age of a woman. Identified genes are responsible for pathogenesis of symptoms in trisomy 21, Turner and Klinefelter syndromes. Diagnostics of chromosomal diseases includes prenatal screening programs and postnatal testing.*

Conclusions. *Cytogenetic variants of Down syndrome are simple complete trisomy 21, translocation form and mosaicism. Trisomy 21 is associated with advanced maternal age. Phenotypic manifestations of Down syndrome are associated with the locus 21q22. The maternal and parental nondisjunction of X-chromosomes in meiosis causes Klinefelter and Turner syndromes. These chromosomal diseases are variants of intersexualism with intermediate chromosomal sex. Down-Klinefelter and Down-Turner syndromes are double aneuploidies. Patients have a Down syndrome phenotype at birth, and signs of Klinefelter and Turner syndromes occur during puberty. Diagnosis of aneuploidy is based on the cytogenetic investigation (karyotyping), DNA analysis, ultrasonography and biochemical markers of chromosomal pathology.*

KEY WORDS: **Down syndrome; Klinefelter syndrome; Turner syndrome; double aneuploidy.**

Introduction

Changes in the number and structure of chromosomes take place in 1% of newborns. They are the cause of 45-50% of multiple birth defects, about 36% of cases of mental retardation, 50% of primary amenorrhea in women, and 10% of sterility in men [1]. Among all cases of birth defects in the life of stillbirths in Ukraine for 2002-2015, almost every 2nd case referred to chromosomal pathology. The main clinical chromosomal syndromes include: Down syndrome (DS) – trisomy 21 – prevalence 1:400-1:1,500 newborns, Klinefelter syndrome (KS) – karyotype 47, XXY with a frequency of 1:500-1,000 newborn boys, Turner syndrome (TS) – karyotype 45,X with a frequency of 1:2,000-5,000 newborn girls [2-6]. Among the viable newborns, 1 of 400 boys and 1 of 650 girls have different forms of aneuploidy by sex chromosomes. KS and TS are most common among them [7].

Double aneuploidy is a rare chromosomal abnormality detected after birth since most reported cases are presented in the form of one lethal aneuploidy and end in early miscarriage. It is extremely rare to find living newborn cases with double autosomal trisomy or autosomal trisomy with sex chromosome monosomy or trisomy [8]. The most frequent co-occurrence is that of DS and KS (coincidence 0.098%), because they are common and relatively well tolerated chromosome abnormalities in humans [9].

The frequency of mutations that causes chromosomal diseases does not depend on race or country. The dynamics of the spread of chromosomal diseases over the past two decades in the world has changed due to the discovery of new cases of double aneuploidy in viable newborns. Early prenatal diagnosis of trisomy 21 as well as postnatal aneuploidy by sex chromosomes is urgent.

Basic research on aneuploidy is conducted using genomic technologies to identify a correlation between genotype and phenotype in chromosomal diseases and to understand its pathogenesis. For diagnosis of hereditary dise-

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ases, the medical genetics methods are used, in particular cytogenetic, genealogical, dermatoglyphics, biochemical, and DNA molecular analysis. The cytogenetic method (karyotyping) is widely used for diagnosis of human chromosomal diseases. It is important to be aware of diagnostic possibilities of prenatal testing for early detection of fetal chromosomal pathology such as screening programs for women at early pregnancy. The more deeply the nature of human heredity is analyzed, the more it is realized regarding the methods of diagnostics, treatment and prevention of diseases.

The aim of the research is to study the cytogenetic mechanisms of occurrence, pathogenesis of changes in the phenotype and medical genetic counseling for patients with DS, KS and TS.

Methods

A literature review by the keywords "Down syndrome", "Klinefelter syndrome", "Turner syndrome", "double aneuploidy" for the period of 2000-2020 was carried out.

Review

According to the literature, in 1866 English physician John Landon Down identified a group of patients with dementia and peculiar somatic abnormalities and called the disease "Mongoloid idiopathy". The hypothesis of hereditary origin of the syndrome was considered questionable, although in 1932 the ophthalmologist and geneticist Waardenburg suggested that the basis of the pathology may have been "chromosomal insufficiency" [2-3]. Despite this, the cause of this syndrome was clarified only in 1959 by a French scientist Lejeune. He stated that in the karyotype of those patients there were 47 chromosomes, and a small acrocentric chromosome in the group G (21 or 22) was superfluous. As a result of development of the fluorescence chromosome identification method in 1971, it was possible to differentiate 21 of 22 chromosomes and establish the true karyotype in DS. The incidence of patients' birth with trisomy 21 is not affected by sexual, racial, geographical or population differences. The incidence of children with DS depends on the age of the mother (Table 1) [1]. More than 80% of children with trisomy 21 are born to women under 35 years of age [3]. Hence, prenatal screening and diagnosis of trisomy 21 for a fetus are important for pregnant women aged 35 years and older [10].

Cytogenetic variants of DS are diverse. However, the main part (94-95%) is the cases of simple complete trisomy 21 as a consequence of the nondisjunction of chromosomes to the opposite poles of the cell in the anaphase I or anaphase II of the meiosis. The contribution of maternal nondisjunction is 95 %, and the father's only 5 %. No dependence on father's age was found in cases of DS where the additional chromosome originated from father. About 75% of maternal nondisjunction occur during meiosis I, and 25% – during meiosis II, but about 25% of paternal nondisjunction occur during meiosis I, and 75% – during meiosis II [14]. As a result, gametes (oocyte or sperm) have 22 chromosomes (-21) or 24 chromosomes (+21). When a normal gamete that has 23 chromosomes fuses with gametes with 24 (+21) chromosomes, then a zygote 47,+21 is formed, and a child with DS may be born. The karyotype of patients with this will be 47,XX,+21 or 47,XY,+21 [11]. About 2-5% of patients with DS have mosaic forms due to the mitotic nondisjunction of the chromosomes after fertilization (post zygotically), in which one part of the cells has a normal amount of chromosomes (46), and the other is aneuploid (47). The karyotypes of patients with 47,XX,+21/46 or 47,XY,+21/46 [11, 13]. The frequency of gonadal mosaicism in trisomy 21, according to some researchers, is actually larger. Available for tissue analysis (blood lymphocytes, skin fibroblasts) have a low mosaic level, while an abnormal line is present in gonads. Study of QRQ polymorphism of chromosomes in 151 families of Down children showed that in 8 families there is gonadal mosaicism in trisomy 21, with all cases of mothers younger than 35 years old. The frequency of carriage of mosaicism in young families was 6.5% (8/123). Approximately 4% of patients with DS have a translocation form. The patients have a diploid chromosome set, but their morphology is disturbed. This form occurs, as a rule, as a result of the Robertson translocation of surplus

Table 1. The risk of childbirth with Down syndrome in women of different age groups

Age of a woman at the time of childbirth, years	Empirical risk of having a baby with Down syndrome
till 19	1:1640
20-24	1:1986
25-29	1:1319
30-34	1:603
35-39	1:217
40-44	1:84
45	1:31

21 chromosomes between the acrocentric cells (D/21 and G/21). 25% of translocation forms are inherited from native carriers, and 75% of the translocations are formed *de novo* [14-15]. The translocation form of the DS is not phenotypically different from the classic trisomy 21. The occurrence of this pathology does not depend on the age of parents, while the parents can be phenotypically healthy carriers of translocation (45,-21,t(15q21q)). Consequently, people with a well-balanced translocation have a high risk of having a child with translocation form of DS (1:3). At normal karyotype of parents, the risk of having a baby with trisomy 21 corresponds to a simple trisomy. If a patient has a translocation variant of DS, then the parent's karyotype must be explored. In young parents, who have children with DS, balanced chromosomal aberrations (1.95 %) are found in 8 times more often than in the other population. When translocation type 13/21 is revealed in the father, the risk of birth of a sick child is 2.4%, and in the mother – about 10%. If one of the parents has a translocation type 21/21, the risk of having a baby with Down syndrome is 100%. About 10% of mothers of the children with DS have mosaicism. In the case of parents' mosaicism, genetic risk should be considered about 30% [1]. Other forms of trisomy 21 are 1% of DS. They can be caused by: a) a terminal rearrangement of chromosome 21 around the telomeric region, the final chromosome having two centromeres and satellites on both ends; b) as a component of a double aneuploidy (for example, 48,XYY,+21 or 46,X,+21) [14, 16].

Chromosome 21 is the smallest human chromosome and contains 200 to 300 genes. Analysis of the chromosome revealed 127 established genes, 98 predicted genes and 59 pseudogenes [14]. The most acceptable theory for the pathogenesis of trisomy 21 is the gene-dosage hypothesis, which declares that all changes are due to the presence of an extra copy of chromosome 21 within region *21q22*. Molecular studies are aimed at determining the region of 21 chromosomes, which would code for phenotypic manifestations of DS. These gene products include morphogens, cell adhesion molecules, components of multi-subunit proteins, ligands and their receptors, transcription regulators and transporters. In particular, it has been found that the *DYRK* gene (*21q22.13*) is responsible for mental retardation. Surplus of this gene in mice causes defects in learning and memory. The third copy of the other *APP* gene (*21q21.2*|*21q21.3*) encoding the β-amyloid synthesis causes Alzheimer's syndrome in the patients with trisomy 21 of over 40 years of age. If as a result of mutation this gene in the patients with DS is absent, the signs of Alzheimer's syndrome are not manifested [1, 3]. *Gart-gene* is also detected in the 21 chromosome which in trisomy causes an increased level of purines in the blood of patients that is one of the causes of mental retardation [17]. By the results of expression of this gene you can control the occurrence of DS even in the embryonic period.

The complex of congenital abnormalities specific for DS causes the clinical picture "all children from one family". Pregnancy, from which patients are born, often is accompanied by toxicosis, the risk of miscarriage. In sick mothers there is an unfavorable obstetric anamnesis (miscarriages, stillborn). The average length of pregnancy is slightly lower than normal. Children with DS are born with moderate pronounced hypoplasia (mass 8-10% below average values) [18]. Craniofacial dysmorphia is manifested by brachycephaly (81%), flat occiput (78%), flattened face, flat bridge of nose (52%), small short nose (40%), large fissured tongue (50%), open mouth (65%), hypoplasia of the upper jaw, high palate (58%), small lowly placed deformed ears, mongolism, epicanthus (80%). Incorrect growth of the teeth, diastema, transverse striae on the lips are typical. In 68% of patients, there are spots of Brushwild (light spots on the iris), 32.2% – cataract. In many patients with trisomy 21, there is a short neck (45%), newborn neck folds, broad fingers and palms, broad toes (70%) [1]. Approximately 40-50% of the affected children have congenital heart diseases (CHD), one of the major causes of morbidity and mortality [19-20]. In Western European countries and the USA, endocardial cushion defect (43%), which results in ventricular septal defect (VSD) (32%), secundum atrial septal defect (ASD) (10%), tetralogy of Fallot (6%) and isolated patent ductus arteriosus (4%) are present in this group of patients [14]. In Asia, isolated VSD has been reported to be the most common cardiac defect (40%) [21]. A study from Korea showed that ASD was the most common defect accounting for 30.5 % of DS, followed by VSD (19.3%), patent duct arteriosus (17.5%) and atrioventricular septal defect (9.4%) [22]. The second type of ASD was the most common cardiac lesion in Latin America [23-24]. In Libya, the most common isolated cardiac lesion was the ASD, found in 23% of the patients [25]. Molecular studies of rare individuals with CHD and partial duplications of chromosome

21 established candidate gene *DSCAM*, which was manifested in the heart during cardiac development [26]. About half of the atrioventricular septal defects AVSDs occur in the patients with DS [27]. In the patients with DS and complete AVSD potentially damaging variants in six genes: *COL6A1*, *COL6A2, CRELD1, FBLN, FRZB, GATA5* were identified [28].

Immune problems are also typical in the form of immunodeficiency. Laboratory abnormalities can be revealed in both humoral and cellular immunity. DS is manifested by high incidence of leukemia. However, the children with DS have a 20-fold greater risk for acute lymphoid leukemia and a 50-fold higher risk for myeloid leukemia compare to the children without DS [29]. Development of DS brain is associated with reduction in the neuronal number and abnormal neuronal differentiation. It has been previously reported that DS neuron degenerate subsequently and undergoes apoptosis [4]. The mental retardation with full trisomy 21 is found in almost all patients, and it is basically oligophrenia at the stage of imbecility (65-90%). The average IQ of children 10 years old and over is only 24. In a mosaic variant of DS, moderate mental retardation is much more common, and normal intelligence is also possible. In the United States DS is 10% of all cases of mental retardation. DS is associated with epilepsy in 10% of cases. Hearing loss and anomalies of the vision organs is often observed in child patients, in particular, cataracts in 66% of the children over 8 years of age [1]. The patients with DS gain more weight compare to other population, and most adults with DS are overweight. Antithyroid antibodies predispose to disturbance of gametogenesis and nondisjunction of the 21 pair of chromosomes. The hypofunction of the thyroid gland in parents is a risk factor. Nowadays, cardiac surgery, vaccinations, antibiotics, thyroid hormones, leukemia therapies, and anticonvulsive drugs (e.g, vigabatrin) have significantly improved the quality of life of individuals with DS. Actually, life expectancy that was hardly 30 years in the 1960s is now increasing – more than 60 years of age [11, 30]. Oral care by dentists for the children with DS from the first months of life involve palatal plates that aim at activating and stimulating different functions, mainly for the orofacial motor function and expression, including language. This is complementary for that of speech therapists, physiotherapists, psycho-motor therapists, and pediatricians [31-32].

Reproductive capacity in DS is described in women, with 40% being ovulatory disorders. Men with this syndrome are sterile. Women with DS produce half normal eggs and half with 24 chromosomes. Consequently, the probability of zygote with trisomy 21 in women with DS is 50%. As about 75% of these pregnancies are completed with spontaneous abortion, the risk to birth of a sick child is less than 50%. Trisomy 21 among aborted embryos is found at a frequency of 1:40. The cytogenetic examination in children with DS is indicated with suspicious of trisomy 21 and with clinically established diagnosis, as the patient's cytogenetic characteristics from parents and their relatives are needed to predict the health of future children [1].

Diagnostics of DS includes prenatal and postnatal testing. For pregnancies, the high risk of DS is evaluated by fetal sample analysis after invasive chorionic villus sampling (CVS) and amniocentesis, and by laboratory techniques such as conventional karyotype analysis, Fluorescence in Situ Hybridization, Quantitative Fluorescence-Polymerase Chain Reaction, Multiplex Ligation Probe Assay and Array Comparative Genomic Hybridization, which are common techniques used for prenatal diagnosis of DS and each of them presenting with advantages and disadvantages. There is also a noninvasive technique for detection of trisomy 21 by analysis of extracted cell-free fetal DNA screening from maternal plasma samples [14]. The most common screening method contains the measurement of a combination of factors: advanced maternal age, multiple second trimester serum markers and second trimester ultrasonography. The first reported marker associated with DS was the thickening of the neck area. 40-50% of the affected fetuses have a thickened nuchal fold measuring ≥6 mm in the second trimester. When screening by nuchal translucency (NT), about 83% of trisomy 21 pregnancies were identified in the first trimester. Later, it was revealed that screening by a combination of maternal age, NT and bi-test (PAPP-A with second trimester β-hCG) or tri-test [alpha-fetoprotein (AFP), estriol and free β-hCG] has a potential sensitivity of 94% for a 5% false positive rate [4, 33]. However, Ruei-Yu Lan et al, reported about multicenter study of 29,137 cases that enrolled the chromosomal abnormality screening from Taipei city. The mean maternal age of screen-positive group was 34±4.2 years old. The first trimester had 891 cases screening positive with a detection rate of 97.5% for fetal trisomy 21 and false

positive rate of 3.5%. Second-trimester had 334 cases screening positive, the detection rate and false positive rate were 33.3% and 6.4% respectively for trisomy 21 [10].

More recent advances in genomics and related technologies have resulted in development of a noninvasive prenatal screening (NIPS) test using cell-free fetal DNA sequences isolated from a maternal blood sample. Almost 5-10% of DNA in maternal serum are of fetal origin. Fetal trisomy detection by DNA-analysis from maternal blood has been done using massively parallel shotgun sequencing (MPSS). By next generation sequencing platforms, millions of amplified genetic fragments can be sequenced in parallel. MPSS detects higher relative amounts of DNA in maternal plasma from the fetal trisomy chromosome compare with reference chromosomes. Platforms differ according to whether amplified regions throughout the genome, chromosome-specific regions, or single nucleotide polymorphisms (SNPs) are the targets for sequencing [34]. Although studies are hopeful and exhibit high sensitivity and specificity with low false-positive rates, there are drawbacks to NIPS. Specificity and sensitivity are not consistent for all chromosomes; this is due to different content of cytosine and guanine nucleotide pairs. False-positive screening results take place because the sequences derived from NIPS are derived from the placenta, like in CVS they may not reflect the true fetal karyotype. Therefore, currently invasive testing is recommended for confirmation of a positive screening test and should remain as option for patients seeking a definitive diagnosis. NIPS began as a screen for only trisomy 21 (T21) and rapidly developed to include other common aneuploidies for chromosomes 13 (T13), 18 (T18), X, and Y [35, 36]. Amniocentesis is the most conventional invasive prenatal diagnostic method accepted in the world. It is mostly performed to acquire amniotic fluid for karyotyping from 15 weeks onwards. Actually, amniocentesis performed before 15 weeks of pregnancy is referred to as early amniocentesis. CVS is usually performed between 11 and 13 weeks of gestation and includes aspiration or biopsy of placental villi. Amniocentesis and CVS are quite reliable but increase the risk of miscarriage up to 0.5 to 1% compare with the background risk [33].

A number of European countries have established a network of OSCAR clinics (One Step Clinic for Evaluation of Risk for Fetal Abnormalities). Clinics of "one visit" allow calculating the individual risk of a child's birth with chromosomal pathology regarding of the age of pregnancy, ultrasound and biochemical markers of chromosomal pathology. In particular, among the biochemical markers in the first trimester, the content of plasma protein-associated protein (PAPP-A) and β-chorionic gonadotropin (β-hCG) levels are determined [18]. For non-invasive prenatal diagnosis, studies of fetal trophoblastic cells from the cervical canal of women in the first trimester of pregnancy are also used [37]. Constantly conventional karyotype from peripheral blood in postnatal period is performed to confirm diagnosis for all patients suspected with DS [14].

Polysomy by sex chromosomes has less significant pathological manifestations than aneuploidy by autosomes. It may be explained by the fact that the Y chromosome contains little genes, and additional X chromosomes may be heterochromatic. Genetic intersexualism is the result of sex chromosome aneuploidies. TS and KS are variants of intersexualism with an intermediate sex [7].

TS (45,X) is a single form of monosomy in the liveborns. Monosomy X was discovered by Henry Turner in 1938 (earlier, in 1930, the description was made by Otto Ullrich). Cytogenetic syndrome 45,X was discovered by SE Ford in 1959 [5]. Clinically TS manifests itself in three directions: 1) hypogonadism, underdeveloped genital organs and secondary sexual characteristics; 2) congenital defects; 3) short stature. TS is one of the clinical forms of sexual differentiation disorders – agenesis with somatic malformations. In the reproductive system there is a lack of sexual glands (agenesis of gonads), hypoplasia of the uterus and fallopian tubes, primary amenorrhea, inadequate hair follicles in the pubic and axillary areas, underdevelopment of the mammary glands, deficiency of estrogens, and excess of pituitary gonadotropins. In children with TS, congenital malformations of the heart, vessels and the kidneys make up to 25%. In newborns, the neck is short with excess skin and wing-like folds, with low neck hair growth. These patients have lymphatic edema of the feet, legs, hands and forearms. At school age, especially at the puberty period, there is a lag in the growth and development of secondary sexual characteristics. In 16-50% of cases the patients with TS are mentally retarded, prone to the lymphedema and Hashimoto's thyroiditis. It is established that if a TS patient received an X-chromosome from their father, they would have a higher IQ and better adaptation in so-

ciety than that received from the mother's sex chromosome. Chromosomal anomalies in women with TS are quite different: 50% of these patients have 45,X karyotype in blood lymphocytes, 30-40% are mosaics in most cases with a karyotype 45,X/46,XX, less than 45,X/46,XY; 45,X/47,XXX. Among liveborn limited placental mosaicism is often observed. The more normal cells a fetus has the greater chance of its survival. About 10-20% of patients with TS have structural changes in the X-chromosome, which include deletion of short or long arms (46,X,Xp-; 46,X,Xq-), isochromosomes [46,X,i(Xq); 46,X,i(Xp)], ring chromosomes [46,X,R(X)]. These varieties of chromosomal aberrations stipulate for the phenotype of patients. Molecular studies have shown that 60-80% of monosomies X are due to the disappearance of the paternal sex chromosome that occur either in early mitosis during embryogenesis or in the process of meiosis. A repeated risk of birth of a sick child with TS in a family with normal parents karyotype does not exceed 1% [7]. Molecular analysis also allowed precise determining of specific genes responsible for the phenotype of TS. For example, the mutation in the *SHOX* gene causes low growth. This gene is localized in the distal segment of the X chromosome (Xp22.33) and in the short arm of the Y chromosome. Consequently, this gene undergoes normal transcription being in two copies of a man and a woman. So, as the patients with TS have only one copy of this gene, they have a high probability of short stature development. According to Linglart et al. [38], hormone reposition in the patients with TS may reduce likelihood of short stature development. Webbed neck, lymphedema or coarctation of the aorta in infancy should prompt a peripheral blood karyotype to rule out TS [39]. The standard 30-cell karyotype is recommended and can detect 10% mosaicism with 95% confidence [5]. Multiple sequences adjacent to the Y-centromere should be amplified using polymerase chain reaction techniques to detect cryptic Y-material. When Y-chromosome material is present in the standard karyotype or on such additional testing (incidence of 5-12%), prophylactic gonadectomy is still recommended by expert consensus, albeit at a lower quality of evidence, due to an increased risk (around 10%) of gonadoblastoma [39-40]. In TS fetal ovaries develop normally in the early stages of embryogenesis, but the absence of a second X-chromosome leads to an accelerated loss of oocytes within 2 years. Spontaneous puberty has been reported in 14% of TS patients with monosomy X and up to in one third of patients with mosaicism [5]. Young TS women with normal ovarian function should be counseled about fertility preservation options. Gonadotropins (especially follicle-stimulating hormone) should be monitored annually starting at about 11 years of age to confirm hypergonadotrophic hypogonadism prior to pubertal induction [6]. Anti-Müllerian hormone (AMH) and inhibin B measurements have also been shown to predict ovarian insufficiency when found to be low, and AMH is perhaps the best indicator of ovarian reserve [41]. However, the majority of the girls with TS require induction of puberty and estrogen/progestin replacement therapy to achieve adequate breast development, uterine maturation and peak bone mass. Transdermal 17-β estradiol (TDE) is now the preferred treatment starting at age around 11-12 years old. Compared with oral estrogens, TDE is thought to be more physiologically delivery since it will avoid the first-pass effect in the liver with improved bioavailability. A recent meta-analysis showed improved whole-body bone mineral density, fasting glucose and total cholesterol with TDE therapy compare with oral estrogens [42].

KS is the most extensive major anomaly of sexual differentiation in men. The disease is described in 1942 by Harry Klinefelter in males with sexual retardation. In 1959, Jacobs and Strang showed the presence in these patients of a pathological karyotype. The most common cause of KS is nondisjunction of XX homologues for meiosis. Meiotic non-disjunction events causing KS are inherited maternally in 50-56% of cases (36% occur in 1st and 20% in 2nd meiosis), and paternally in 44-50% (100% 1st meiosis) [43, 44]. With the age of the mother, as with DS, the probability of birth of a baby with this syndrome increases [1]. In 40-year-old mothers, ill children are born by 2-3 times more often than in 30-year-olds. Preliminary, it is established that half of cases with a karyotype 47, XXY end with spontaneous abortions. Mosaic among liveborn is observed in 15% of cases [7]. The presence of the Y chromosome determines the formation of a male phenotype. Genetic imbalance due to excess X-chromosome is manifested clinically during puberty as underdevelopment of the testicles and secondary male sexual characteristics. The presence of an extra chromosome can cause higher levels of gene expression and gene products in amounts affected by regulation at different levels, pro-

tein degradation and modification [45]. Also, the interaction pattern of the proteins encoded by the additional chromosome can influence the severity of a trisomy. In the case of KS, X chromosome inactivation (XCI) counterweights the extra amount of X chromosome genes (about 2000). Still, roughly 15 % of X-linked genes escape XCI, including genes in the two pseudoautosomal regions (PARs). A total of 22 X chromosome genes were deregulated in KS compare to the controls: 16 were upregulated (*AKAP17A, ASMTL, CSF2RA, EIF1AX, EIF2S3, GPR82, GTPBP6, IL3RA, PLCXD1, PPP2R3B, PRKX, RP11-EPO6O15.3, SEPT6, SLC25A6, TMSB4X* and *XIST*) and six were down-regulated (*BEND2, BEX1, COX7B, FOXO4, NHS and TFE3*). Some of these genes were expressed from PAR1: *AKAP17A, ASMTL, CSF2RA, GTPBP6, IL3RA* and *PLCXD1*, but no differentially expressed genes were observed encoded from PAR2. XCI compensates extensively for the extra X chromosome gene dosage in KS males and probably increases their chances of survival compare to other aneuploidies [17].

Thus, in the patients with KS have a high stature, eunuchoidism, narrow arms and wide pelvis, subcutaneous fat development by female type, absent hair growth on the face, in the axillary areas and in the area of the pubis, sparse hair, underdeveloped testicles and their endocrine function (production of androgens) is reduced. The prevalence of androgen deficiency in the patients with KS is 79%. The patients in most cases are sterile (due to azoospermia, oligospermia). Among men, who have infertility, more than 10% have an additional X-chromosome. Approximately one in three patients with this syndrome has gynecomastia and an increased risk of breast cancer development. This risk can be reduced by having a mastectomy. Gynecomastia and obesity with fat deposits for the female type are a result of relative hyperestrogenemia [7]. In the KS patients this may lead to an increased incidence of female predominant autoimmune disorders [46].

The system biology approaches together pointed to novel aspects of KS phenotypes including perturbed JAK-STAT pathway, dysregulated genes important for disturbed immune system (IL4), energy balance (POMC and LEP) and erythropoietin signaling in KS. Sex chromatin is detected in 80% of the patients with KS. Modified laboratory parameters: decreased testosterone in the blood and urinary excretion of 17-ketosteroids; an increase in the amount of estrogen in the plasma, as well as their excretion with urine; high levels of gonadotropins in plasma, especially follitropin, are observed. Biopsy of testicles should be used due to the inaccuracy of laboratory parameters (hyalinosis of the walls of seminal tubules, aplasia of the generative epithelium, aspermia). Usually KS males present a normal growth hormone – insulin-like growth factor IGF-I axis and increased luteinizing hormone and follicle-stimulating hormone levels due to their androgen deficiency [17]. However, early diagnosis of sex chromosome syndromes may provide better development and quality of life for patients with these disorders. Samango-Sprouse et al. [47] observed significant advantages in the cognition, language, intellectual, and psychomotor functions of the patients with KS treated with hormone therapy at 3-6 years of age compare to a group of untreated patients.

The double aneuploidy 48,XXY,+21 was first described in 1959, and the incidence is reported to be 0.4 to 0.9 per 10,000 male births [9, 47, 48]. Most commonly, double aneuploidy occurs when two meiotic nondisjunction events happen from the same or different parental origins. The coincidence rate of DS and KS in the same patient is 0.098 % in newborns, and it has been found to dependent on parental age. Advanced maternal age is one of the main risk factors for double aneuploidy, as it is for single chromosomal aneuploidies caused by parental nondisjunction during gametogenesis [49-51]. The nonrandom aspect of double aneuploidy provides evidence that a hereditary predisposition to nondisjunction exists, with one chromosomal imbalance increasing the risk of another to occur [52]. The phenotype of 48,XXY,+21 double aneuploidy is characterized by classical DS features in children, who then develop additional KS symptoms from the age of puberty [9, 52]. Since the sexual development primarily depends on the presence of the Y chromosome, the 48,XXY,+21 individuals have male phenotype. KS is a form of primary testicular failure with testicular hypotrophy and elevated gonadotropin plasma levels, and it is the most common form of male hypogonadism [53]. In addition, the spectrum of different mental problems could be wider, and their risk could be higher in this group of patients. In cases of combined DS and KS the incidence of congenital heart disease could be higher compare to the isolated forms of these aneuploidies. Sevcan Tug Bozdogan and Atil Bisgin reported of Down-Klinefelter syndrome with accompanying both heart defects and hypothyroidism [49].

The combination of DS and TS is very rare (1 in 2,000,000) [15, 54]. Evren Gumus observed a two-year old girl with karyotype from the peripheral lymphocytes using G-bandings "mos45,X[41]/47,XX,+21[59]". She had stigmata's such as up slant palpebral fissures, brachycephaly and epicanthus, renal hypoplasia and hepatomegaly. In this case, at early ages, the clinical picture is completely compatible with the autosomal chromosome aneuploidy, but the phenotypic effects of TS will become dominant in post pubertal period [15].

Therefore, Down-Klinefelter and Down-Turner syndromes are rare chromosomal abnormalities. A patient present with physical characteristics of DS at birth but KS or TS features during puberty. It is important to counsel families about recurrence risk for future pregnancies. A clinical geneticist evaluation is also recommended.

Conclusions

Cytogenetic variants of Down syndrome are simple complete trisomy 21, translocation form and mosaicism. Trisomy 21 is associated with advanced maternal age. Phenotypic manifestations of Down syndrome are associated with the locus *21q22*. The maternal and parental nondisjunction of X-chromosomes in meiosis causes Klinefelter and Turner syndromes. These chromosomal diseases are variants of intersexualism with intermediate chromosomal sex. Down-Klinefelter and Down-Turner syndromes are double aneuploidies. Patients at birth have a Down syndrome phenotype, and signs of Klinefelter and Turner syndromes appear during puberty. Diagnosis of aneuploidy is based on the cytogenetic investigation (karyotyping), DNA analysis, ultrasonography and biochemical markers of chromosomal pathology.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Bihunyak T.V. – conceptualization, writing – original draft; *Bondarenko Yu.I.* – supervision; *Kulyanda O.O.* – writing – review and editing; *Charnosh S.M.* – formal analysis; *Sverstiuk A.S.* – software; *Bihuniak K.O.* – validation.

ХРОМОСОМНІ ЗАХВОРЮВАННЯ В ПАТОЛОГІЇ ЛЮДИНИ (огляд літератури)

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

Вступ. *Хромосомні захворювання є причиною 45-50 % множинних вроджених вад. Основні дослідження мутацій – це використання геномних технологій для виявлення співвідношення генотипу та фенотипу при анеуплоїдіях та розуміння їх патогенезу.*

Мета. *Метою дослідження є вивчення етіології, патогенезу симптомів та методів діагностики пацієнтів із синдромами Дауна, Клайнфельтера, Тернера та подвійними анеуплоїдіями 21 та статевих хромосом.*

Методи. *Був проведений пошук за ключовими словами "синдром Дауна", "синдром Клайнфельтера", "синдром Тернера", "подвійна анеуплоїдія" публікацій за період 2000-2020 років.*

Результати. *Синдроми Дауна, Клайнфельтера та Тернера є найпоширенішими анеуплоїдіями серед життєздатних новонароджених. Частота мейотичних нерозходжень, які спричиняють ці анеуплоїдії, зростає з віком жінки. Ідентифіковані гени, які відповідають за патогенез симптомів при синдромах трисомії 21, Тернера та Клайнфельтера. Діагностика хромосомних захворювань включає програми пренатального скринінгу та постнатальне тестування.*

Висновки. *Цитогенетичні варіанти синдрому Дауна – це проста повна трисомія 21, транслокаційна та мозаїчна форми. Трисомія 21 асоціюється із збільшенням віку матері. Фенотипові прояви синдрому Дауна пов'язані з локусом 21q22. Материнське та батьківське нерозходження Х-хромосом при мейозі спричиняє синдроми Клайнфельтера та Тернера. Ці хромосомні захворювання є варіантами інтерсексуалізму з проміжною хромосомною статтю. Синдроми Дауна-Клайнфельтера та Дауна-Тернера є подвійними анеуплоїдіями. Пацієнти при народженні мають фенотип синдрому Дауна, а ознаки синдромів Клайнфельтера та Тернера з'являються у період статевого дозрівання. Діагностика анеуплоїдій грунтується на цитогенетичному дослідженні (каріотипуванні), аналізі ДНК, ультрасонографії та біохімічних маркерах хромосомної патології.*

КЛЮЧОВІ СЛОВА: **синдром Дауна; синдром Клайнфельтера; синдром Тернера; подвійна анеуплоїдія.**

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STAPHYLOCOCCUS WARNERI CLINICAL ISOLATE SUSCEPTIBILITY TO ANTIBIOTICS AND ITS MODIFICATION BY EMOXYPINE

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Background. *S. warneri is a common commensal organism, but it can cause serious infections. One of the ways to increase a susceptibility of this microorganism to antibiotics is their combining with adjuvant remedies.*

Objectives. *The aim of the research is to study the susceptibility of S. warneri clinical isolate to antibiotics and its modification by emoxypine - 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride.*

Methods. *Samples of purulent wound exudation were obtained from a patient with infective complications after a bone fracture and osteosynthesis by metal plates. Susceptibility of S. warneri clinical isolate to antibiotics and their combinations with emoxypine (1000 μg/disk) was studied by a standard disc diffusion technique. In the case of microbial resistance, the susceptibility of the investigated isolate to such antibiotic was determined by a serial broth dilutions method without or with emoxypine and evaluated according to the minimum inhibitory concentration (MIC).*

Results. *By the disk diffusion method, S. warneri isolate was susceptible to all tested antibiotics, except chloramphenicol. The growth inhibition zones also were formed around disks containing emoxypine, but the susceptibility of S. warneri to this agent was low. Applying of emoxypine on the disks with antibiotics resulted in the increase of S. warneri growth inhibition in all cases, except using the amikacin, gentamicin, and fusidic acid. The most significant changes were evidenced for a composition chloramphenicol / emoxypine. Using chloramphenicol alone in the liquid medium, the MIC was over a cut-off point. Adding of emoxypine (209 μg/ml) decreased the MIC of the antibiotic and restored the susceptibility S. warneri to chloramphenicol.*

Conclusions. *The susceptibility of S. warneri clinical isolate to antibiotics can be increased by combining with emoxypine, which among other overcomes the resistance of the studied microorganism to chloramphenicol.*

KEY WORDS: ***S. warneri*, susceptibility, antibiotics, 2-ethyl-6-methyl-3-hydroxypyridine (emoxypine).**

Introduction

Coagulase-negative staphylococci (CoNS) were taken as insignificant contaminant earlier, but now they are regarded as a cause of many multidrug resistant infections. The significant change in the patients profile, that is an increased number of premature newborns, elderly patients, chronically ill patients, and immunocompromised patients along with greater use of indwelling or implanted foreign bodies has made CoNS a predominant nosocomial pathogen [1]. *S. warneri* is a catalase-positive, oxidase-negative, and CoNS, and is a common commensal organism found as part of the skin flora in humans and animals [2]. This pathogen can cause abortion in domestic animals and humans [3]. In humans, it causes discitis, urinary tract infections, and meningitis [4, 5, 6]. Orthopedic infections due to *S. warneri* also have been reported [7, 8]. Over the last two decades, similarly to other CoNS species, *S. warneri* has been reported as a new emerging pathogen, capable of causing serious infections usually in association with the presence of implant materials [9, 10]. Very often *S. warneri* strains isolated from clinical material are resistant to one-two antibiotics or even all tested antimicrobials (to 18% of clinical isolates) [11].

One of the ways to increase a susceptibility of the microorganism to antibiotics is their use in a combination with adjuvant remedies. Synthetic antioxidant 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride (emoxypine) is used in clinic, has its own antimicrobial activity and is able to enhance microbial susceptibility to antibiotics in the experiments with etalon strains of microorganisms [12, 13, 14]. For implementation of these experimental data into the anti-infective therapy, it is interesting to evaluate the influence of 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride on the clinical isolate of *S. warneri* as a representative of CoNS.

The aim of the research is to study the susceptibility of *S. warneri* clinical isolate to anti-

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biotics and its modification by 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride (emoxypine).

Methods

Some samples of purulent wound exudation were obtained from a 57-year old female patient with infective complications developed on the 7th day after the bone fracture and osteosynthesis by metal plates without subsequent antibiotic therapy. It was used for microbiological assessments and diagnosis. The patient has given an informed consent for sampling of wound exudation and the use of these samples for investigation. After collection, the sample was transferred to a microbiological laboratory in less than 1 hour. Bacteriological culture was collected in sterile ball vials. Gram staining was made on the first day. Cultivation was carried out in thioglycolic medium manufactured by the Mechnikov Ilya Institute of Vaccines and Serums. 1.5% of agar "Dyfko", 5% of blood and 0.5-1% of yeast hydrolysate was added to promote growth of microorganisms, followed by isolation of pure cultures of facultative anaerobic bacteria in presence of 5-10% CO_2. The medium was observed daily for microbial growth. Final identification was performed by the automatic bacteriological analyzer Vitek 2 Staphylococcus cards (Biomérieux®, France). The research was performed in the Medical Laboratory BRight-Bio (License AB No. 526132, dated February 4, 2010) and partly in the Research Laboratory of the Department of Microbiology, Virology and Immunology of Ukrainian Medical Stomatologcal Academy. The experiments were approved by the Commission on Bioethics of Ukrainian Medical Stomatologcal Academy.

Susceptibility of the tested isolate to antibiotics was determined using disk diffusion method [15]. The following disks with antibiotics were used: penicillin G (10 μg), oxacillin (30 μg), ampicillin (30 μg), cefoxitin (30 μg), norfloxacin (10 μg) erythromycin (15 μg), tetracycline (30 μg), amikacin (30 μg), gentamicin (10 μg), co-trimoxazole (1.25/23.75 μg), chloramphenicol (30 μg), and fusidic acid (10 μg). All the antibiotic disks were procured by HiMedia Laboratories Pvt Ltd., India. Substance of 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride (emoxypine) was produced by the PLC Lubnyfarm (Ukraine). It was dissolved in water for injections and applied on clean sterile paper disks (HiMedia Laboratories Pvt Ltd., India) or disks with antibiotics to the amount of 1000 μg/disk. These disks were introduced into the disk by diffusion method in the same way as the standard disks with antibiotics. In 18 hours of incubation at 37 °C the Petri plates were examined, and the diameters of inhibition zones were assessed. The results were classified as susceptible or resistant according to the approved guidelines [16]

In the case of microbial resistance, the susceptibility of the investigated clinical isolate to such antibiotic also was determined in a liquid medium without and with emoxypine as adjuvant. Standard macromethod of serial broth dilutions was used [15]. Substance of such antibiotic (chloramphenicol) was produced by the Biopharma Company (Ukraine). Mother solutions contained 312 μg/ml of the antibiotic (0,031% solution), 10000 μg/ml of emoxypine (1% solution), or 312 μg of the antibiotic and 10000 μg of the potential adjuvant per 1 ml. Twofold serial dilutions of the antibiotic, adjuvant and their combination were prepared and dispensed into the laboratory tubes. A growth control tube and a sterility (uninoculated) tube also were prepared. The inoculum was prepared using the direct colony suspension method. An 18-hour old culture of *S. warneri* was grown on blood agar. For inoculation microbial suspension equivalent to 1.0 by McFarland Equivalence Standards, diluted 1/100 in saline, was used, and then the concentration of microorganisms in this suspension was 3×10^{10} CFU/cm^3. Laboratory tubes with macrodilutions were incubated for 24 hours at 37 °C. The amount of growth in the tubes containing the antimicrobial agent was compared with the amount of growth in the growth control tubes. The results were registered visually. The susceptibility of the tested microorganism was evaluated according to the minimum inhibitory concentration (MIC).

Every determination was repeated five times and digital data was processed by the computer programs Statistica for Windows 6.0. The average mean (M) and its standard error (m, SE) were calculated. The lines of variants were checked for normal values. Since in all the cases there was a normal distribution of data, the difference between the groups was evaluated by the Student's t-test.

Results

Gram positive cocci were found in the samples of purulent exudation at the first stage of bacteriological investigation. They were identified as *S. warneri* by automatic microbiological analysis.

Routine disk diffusion method has demonstrated that this clinical isolate is susceptible to majority of antibiotics (Table 1). Zones of growth inhibition around the disks with all antimicrobials, except chloramphenicol, ranged between 24-32 mm that was characteristic to the susceptible strain of CoNS [16]. At the same time, a diameter of growth inhibition zone around a disk with chloramphenicol was less than 18 mm, a cut-off value of susceptibility that testified the resistance to this antibiotic. The growth inhibition zones also were formed around disks containing emoxypine, but their diameters confirmed low susceptibility of *S. warneri* culture to this synthetic antioxidant. For all antibiotics, except chloramphenicol, zones of growth inhibition were more than the same for emoxypine with a credibility from t=11.18 p<0.001 to t=6.47 p<0.005. Diameter of the test culture growth inhibition around disks with chloramphenicol did not differ from this parameter around disks with emoxypine.

Applying of emoxypine on the standard disks with antibiotics resulted in the increase of growth inhibition zones of *S. warneri* test culture as compared to appropriate antibiotic in all cases, except the use of amikacin, gentamicin, and fusidic acid (Table 1). Diameter of the growth inhibition zone for a combination of emoxypine with benzylpenicillin was greater by 9.2 mm (t=4.03, p<0.02), oxacillin – by 9.6 mm (t=7.0, p<0.005), ampicillin – by 6.8 mm (t=2.92, p<0.05), cefoxitin – by 7.0 mm (t=4.43, p<0.02), norfloxacin – by 5.2 mm (t=2.83, p<0.05), erythromycin – by 4.5 mm (t=2.95, p<0.05), tetracycline – by 6.8 mm (t=3.03, p<0.05), co-trimoxazole – by 4.2 mm (t=3.88, p<0.02), chloramphenicol – by 15.4 mm (t=11.32, p<0.001), rifampicin – by 5.5 mm (t=2.78, p<0.05) compare to antibiotic itself. The most significant changes of growth inhibition were observed for a composition of chloramphenicol/emoxypine. Distinguishes between the growth inhibition of the *S. warneri* isolate caused by a combination of amikacin, gentamicin, and fusidic acid with an adjuvant were not significant as compared to the control administered with the standard antibiotic. At the same time, all zones of growth inhibition by combinations of antibiotics with emoxypine were more than the same of the adjuvant drug alone (a credibility from t=22.03, p<0.001, to t=8.10, p<0.002).

Chloramphenicol was only one antibiotic from the tested antimicrobials for which *S. warneri* isolate was resistant in cases of the disk diffusion method and for which effect of emoxypine was the most as compared to other compositions. That is why it also was investigated by a serial broth dilutions method. Results of MIC determination are represented in Fig.1.

MIC was over the cut-off point for CoNS susceptibility, when chloramphenicol was used

Table 1. Zones of growth inhibition of *S. warneri* test culture under the influence of antibiotics and their combinations with emoxypine, M±m (n=5)

Antimicrobial agent	Diameter of growth inhibition zone, mm	
	Antibiotic or adjuvant (emoxypine)	Combination of antibiotic and adjuvant (emoxypine)
Emoxypine (1000 μg)	10.6 ±1.1	–
Benzylpenicillin (10 μg)	27.4±2.0*	36.6±1.1*,#
Oxacillin (30 μg)	28.8±1.2*	38.2±0.6*,#
Ampicillin (30 μg)	26.6±2.0*	33.4±1.2*,#
Cefoxitine (30 μg)	26.8±1.5*	33.8±0.5*,#
Norfloxacine (10 μg)	20.8±1.6*	26.0±0.9*,#
Erythromycin (15 μg)	23.5±1.3*	28.0±0.8*,#
Tetracycline (30 μg)	23.2±1.2*	30.0±1.9*,#
Amikacin (30 μg)	25.0±1.8*	28.0±1.6*
Gentamicin (10 μg)	25.4±1.0*	27.2±1.3*
Co-trimoxazole (1.25/23.75 μg)	19.8±0.9*	24.0±0.6*,#
Chloramphenicol (30μg)	7.8±0.8*	23.2±1.1*,#
Fusidic acid (10 μg)	25.2±1.0*	29.0±1.2*
Rifampicin (5 μg)	28.0±1.4*	33.4±1.9*,#

*Notes: * – p<0.05 compare to growth inhibition zone for emoxypine; # – p<0.05 compare growth inhibition zone for this antibiotic without emoxypine.*

Fig. 1. Minimum inhibitory concentration of chloramphenicol (A) and emoxypine (B) alone and in a combination against *S. warneri* clinical isolate.
*Note. * – p<0.05 as compared with antimicrobial or adjuvant themselves.*

alone [16] (Fig. 1A). With adding of emoxypine MIC of the antibiotic decreased from average 52 µg/ml to 6.5 µg/ml (t=3.47, p<0.05) and the susceptibility of *S. warneri* clinical strain to chloramphenicol restored. At the same time, MIC of emoxypine alone was 416 µg/ml (average); it proved low susceptibility of the studied isolate to this agent (Fig. 1B). A concentration of emoxypine complied with MIC of a composition chloramphenicol/emoxypine was 209 µg/ml, that was 2 times less as compared to MIC of emoxypine (t=3.0, p<0.05).

Discussion

The investigated *S. warneri* clinical isolate was susceptible to the antibiotics of the penicillins, cephalosporins, fluoroquinolones, aminoglycosides, macrolides, tetracyclines, rifampicin groups and steroids, which are recommended for screening of CoNS susceptibility to antimicrobial agents [16]. A high susceptibility of this isolate could be explained by the short history of the hospital treatment and the absence of previous antibiotic therapy in a patient. At the same time, the clinical isolate was resistant to chloramphenicol that was evidenced by a small growth inhibition in cases of disk diffusion method and high MIC in a serial broth dilutions method as compared to cut-off values susceptibility / resistance for CoNS [16]. This can be explained by the nature of chloramphenicol. As it is an ancient microbial metabolite, genetic elements conferring resistance against this drug are retained by and are frequently dispersed in microbial communities [17].

Response of the tested microorganism to emoxypine as a potential adjuvant of antimicrobials was similar to the results of our previous investigations [14, 18]. It was also consistent with other experimental results concerning etalon strains of gram negative rods [12, 13], as well as the retrospective computer prognosis of the biological activity for ethylmettylhydroxypyridine structure that showed a possible antimicrobial activity [19]. Emoxypine's MIC in *S. warneri* test culture was less than this parameter of other CoNS (an etalon strain *S. epidermidis* ATCC 14990) [14]. There are no reference for susceptibility or resistance of bacteria to emoxypine, so we cannot conclude is clinical isolate susceptible to this agent or not, but it is possible to think that *S. warneri* isolates susceptibility to emoxypine is low and the use of this preparation as an adjuvant of antimicrobial therapy will be more perspective. The mechanism of emoxypine antibacterial action is still unclear and the most likely it is due to modification of the bacterial cell membrane typical to antioxidants. Other mechanisms, e.g. protein synthesis inhibition, may also play a part in the antimicrobial action of this 3-hydroxypyridne derivative [19].

Combining of antibiotics with emoxypine led to an increase of growth inhibition zones of *S. warneri* test culture that proved enhance of the clinical isolate susceptibility to these agents. The effect observed was similar to the changes of susceptibility of the etalon strains of *S. aureus* and *E. coli* under the influence of combinations of antibiotics of different classes and relative

preparation: 2-ethyl-6-methyl-3-hydroxipyrydine succinate (mexidol) [14].

In the case of the resistance of *S. warneri* isolate to chloramphenicol, adding of emoxypine restored the susceptibility of the tested microorganism that was registered both in the disk diffusion and serial broth dilutions methods. It is established that resistance to chloramphenicol is mainly present due to the production of a specific inactivating chloramphenicol acetyltransferase, reduced drug uptake or increased drug efflux [17, 20]. That is why it is reasonably that emoxypine effect is caused by its influence on all or some of the mentioned mechanisms, especially on the membrane permeability and efflux pumps because membrane modification is a fundamental property of 2-ethyl-6-methyl-3-hydroxypyridine structure [19].

Overcoming resistance of the clinical isolate *S.warneri* to chloramphenicol occurred with significant MIC of emoxypine in the medium, which is much higher than the maximum concentration of this substance or its salt with succinic acid in the blood plasma after the intravenous administration [21]. Therefore, despite the fact that the description of this phenomenon opens up new possibilities in controlling the susceptibility of CoNS, a matter of the ratio of the concentration of emoxypine in the *in vitro* experiments with a dose of this potential antimicrobial adjuvant in clinic is still unclear. The prospects of the research presented should cover the research of dose/concentration range of emoxypine adjuvant activity.

Conclusions

S. warneri isolate, susceptible to penicillin G, oxacillin, ampicillin, cefoxitin, norfloxacin, erythromycin, tetracycline, amikacin, gentamicin, cotrimoxazole, and fusidic acid, but resistant to chloramphenicol, was isolated from the clinical material. The susceptibility of this clinical isolate to all antibiotics listed, except amikacin, gentamicin, and fusidic acid, can be increased significantly when combined with 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride (emoxypine), which, among other things, restores the susceptibility of the studied microorganism to conventional rates at its concentration of 209 μg/ml.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Nellya O. Bobrova – methodology, investigation; *Galina A. Loban* – project administration, supervision; *Elena M. Vazhnichaya* – conceptualization, writing – review and editing; *Mariia O. Faustova* – formal analysis, writing the initial draft; *Maiia M. Ananieva* – investigation, visualization.

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ЧУТЛИВІСТЬ КЛІНІЧНОГО ШТАМУ STAPHYLOCOCCUS WARNERI ДО АНТИБІОТИКІВ ТА ЇЇ МОДИФІКАЦІЯ ЕМОКСИПІНОМ

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УКРАЇНСЬКА МЕДИЧНА СТОМАТОЛОГІЧНА АКАДЕМІЯ, ПОЛТАВА, УКРАЇНА

Вступ. *S. warneri - звичайний коменсальний організм, але він може спричинити серйозні інфекції. Одним із способів підвищення чутливості цього мікроорганізму до антибіотиків є їх поєднання з ад'ювантними засобами.*

Мета. *Метою роботи є вивчення чутливості клінічного ізоляту S. warneri до антибіотиків та її модифікації етилметилгідроксипіридину гідрохлоридом (емоксипіном).*

Методи. *Зразки гнійного раневого ексудату були одержані від хворої з з інфекційними ускладненнями після перелому кістки і операції остеосинтезу металевими пластинами. Чутливість клінічного ізоляту S. warneri до антибіотиків та їх комбінацій з емоксипіном (1000 мкг/диск) вивчали диск-дифузійним методом. У разі резистентності мікроорганізму чутливість досліджуваного штаму до такого антибіотика визначали методом серійних розведень без або з емоксипіном та оцінювали відповідно до мінімальної пригнічуючої концентрації (МПК).*

Результати. *У диск-дифузійному методі ізолят S. warneri був чутливим до всіх випробуваних антибіотиків, крім хлорамфеніколу. Зони пригнічення росту також утворювались навколо дисків, що*

містять емоксипін, але чутливість S. warneri до цього засобу була низькою. Нанесення емоксипіну на диски з антибіотиками призвело до посилення пригнічення росту S. warneri у всіх випадках, за винятком застосування амікацину, гентаміцину та фузидової кислоти. Найбільш виражені зміни спостерігалися для комбінації хлорамфенікол / емоксипін. При використанні самого хлорамфенікол у рідкому середовищі, МПК була вище точки відсічення. Додавання емоксипіну (209 мкг/мл) зменшило МПК антибіотика та відновило чутливість S. warneri до хлорамфеніколу.

Висновки*. Чутливість клінічного ізоляту S.warneri до антибіотиків можна збільшити, поєднуючи їх з емоксипіном, який, крім іншого, здатний подолати резистентність досліджуваного мікроорганізму до хлорамфеніколу.*

КЛЮЧОВІ СЛОВА: ***S. warneri*; чутливість; антибіотики; 2-етил-6-метил-3-гідроксипіридин (емоксипін).**

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MORPHOMETRIC EVALUATION OF SPERMATOGENIC EPITHELIOCYTES STRUCTURE UNDER THE RUBOMYCIN INFLUENCE IN RATS

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Background. *Over the last 20 years, the quality of men's sperm around the world has deteriorated twice. The causes are poor environment, stress, sedentary lifestyle, prevalence of sexually transmitted infections, obesity, alcohol abuse and various stimulants. The structure and function of the testes, when exposed to various drugs and chemicals, attracts the attention of present researchers.*

Objectives. *The aim of the research was to study the structural changes of the spermatogenic epithelium in testicular damage caused by rubomycin hydrochloride.*

Methods. *The spermatogenic epithelium of the testes of 30 white adult white male rats, which were divided into 2 groups, was morphometrically examined. The 1st group comprised 15 experimental intact animals, the 2nd – 15 rats, in which testicular damage with rubomycin hydrochloride was simulated. Euthanasia of rats was performed by bloodletting under thiopental anesthesia one month after the experiment beginning. Quantitative morphological parameters were statistically processed. In spermatocytes of the 1st P-order, spermatogonia and spermatids of testes, their height, diameter of nuclei, nuclear-cytoplasmic ratio and relative volumes of damaged cells of spermatogenic epithelium were evaluated.*

Results. *It was established that spermatogenic epithelial cells were damaged in the simulated experimental conditions, which was morphometrically confirmed by changes in nuclear-cytoplasmic relations in the 1st, 2nd order spermatocytes, spermatogonia and spermatids. The relative volumes of damaged spermatogenic epithelial cells also increased significantly. The most significant morphometric parameters were altered in spermatids. Thus, the nuclear-cytoplasmic ratio in the studied cells was statistically significantly ($p < 0.001$) increased by 7.4% compared with the similar control morphometric parameter. The relative volume of damaged spermatids in these experimental conditions reached (32.50±0.18) %, which with a high statistically significant difference ($p<0.001$) exceeded the same control value in almost 15.8 times. Optically, histological preparations of the testes showed severe vascular disorders, characterized by dilation and plethora mostly of venous vessels, which pointed to violation of venous drainage of the studied organs.*

Conclusions. *According to the attained research results it has been established that nuclear-cytoplasmic relations are an objective and valuable informative indicator of a functional condition of cells and their structural changes in pathological conditions. When rubomycin hydrochloride is exposed to experimental animals, the most significant changes in nuclear-cytoplasmic relations are found in spermatic spermatozoa.*

KEY WORDS: **rubomycin hydrochloride, testes, spermatogenic epithelium, white rats, morphometry.**

Introduction

It is established that in the structure of infertile marriages, the male factor is 20% [2, 4, 8, 12, 17]. The reason for this is a decrease in spermatogenic and hormonal function of the testes. Often the reason for this is the effect of various toxic factors on the body [2, 13, 14, 19]. In recent years, many researchers are interested in changes in the structure and function of the testes in different physiological and pathological conditions [10, 11, 15, 19].

Rubomycin is an antitumor antibiotic of the anthracycline series with a significant cytostatic effect, which when administered to men can lead to azoospermia (absence of sperm in the ejaculate) [5]. The effect of rubomycin on the structure of the spermatogenic epithelium has been studied insufficiently.

The aim of our study was to investigate the features of structural changes in the spermatogenic epithelium in cases of testicular damage by rubomycin hydrochloride.

Methods

The testicular structures of 30 laboratory adult white male rats, which were divided into 2 groups, were studied by morphometric methods. The 1st group comprised 15 expermental intact animals, the 2nd – 15 rats with simulated

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testicular damage by rubomycin hydrochloride [18]. One month after the beginning of the experiment, euthanasia of experimental animals was performed by bloodletting under conditions of thiopental-sodium anesthesia. Pieces were cut from the testes, which were fixed in 10 % neutral formalin solution, passed through ethyl alcohols of increasing concentration and placed in paraffin. Microtome sections 5-6 mcr thick after dewaxing were stained with hematoxylin-eosin, according to van Gizon, Mallory, Weigert, toluidine blue [7]. Histological micropreparations determined the height of spermatogenic cells, the diameter of their nuclei, nuclear-cytoplasmic ratios, the relative volume of damaged epitheliocytes (VODE) [1, 6, 16]. Morphometry of these structures was performed using a light microscope "Olimpus BX-2" with a digital video camera and application package "Video Test 5.0" and "Video size 5.0". Quantitative values were processed statistically by STATISTIKA software package. The differences between the comparative values were determined by the Mann-Whitney and Student's tests [9]. The studies were performed according to national and international regulations on carrying out experimental tests ("European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments", adopted by the First National Congress on Bioethics (Kiev, 2001)) [8], as well as the Law of Ukraine "On Protection of Animals from Cruelty", dated February 21, 2006.

Results

The attained morphometric parameters are presented in Table 1. A comprehensive analysis of the data showed that most of the studied morphometric parameters of the spermatogenic epithelium changed significantly. Thus, the height of spermatocytes of the 1st order decreased slightly by only 0.65% ($p<0.05$). The diameters of the nuclei of these cells with a high statistically significant difference ($p<0.001$) increased from (5.25±0.03) μm to (5.76±0.04) μm, i.e. by 9.7%. Uneven changes in the spatial characteristics of the nucleus and cytoplasm of spermatocytes of the 1st order led to violations of nuclear-cytoplasmic relations in them. This morphometric index increased by 3.2% ($p<0.05$), which pointed to violation of the studied elements of structural cellular homeostasis [1]. The relative volume of damaged first-order spermatocytes under these experimental conditions with a high statistically significant difference ($p<0.001$) increased almost in 9.7 times.

The morphometric parameters of P-order spermatocytes changed almost similarly under the influence of rubomycin hydrochloride. The height of these cells was almost the same as in the control, the diameters of the nuclei were statistically significantly ($p<0.05$) increased by 2.4%, and the nuclear-cytoplasmic ratio – by 4.9% ($p<0.01$). The relative volume of damaged

Table 1. Morphometric characteristics of cells of spermatogenic epithelium of testes of the experimental animals (M±m)

Cells	Morphometric parameter			
	Height, microns	Diameter of the core, microns	Nuclear-cytoplasmic relations	VODE, %
Control group				
Spermatocytes of the first order	7.65±0.05	5.25±0.03	0.556±0.003	2.20±0.01
Spermatocytes of the second order	5.88±0.04	3.76±0.03	0.410±0.002	2.26±0.02
Spermatogonia	6.12±0.04	4.80±0.03	0.615±0.004	2.15±0.02
Spermatids	4.98±0.03	3.07±0.03	0.389±0.002	2.06±0.02
Experimental group				
Spermatocytes of the first order	7.60±0.05	5.76±0.04***	0.574±0.004*	21.30±0.15***
Spermatocytes of the second order	5.85±0.04	3.85±0.03*	0.430±0.003**	25.60±0,15***
Spermatogonia	6.05±0.04	4.79±0.03	0.628±0.004*	29.80±0.15***
Spermatids	4.92±0.03	3.18±0.02*	0.418±0.002***	32.50±0.18***

*Notes. * – p<0.05; ** – p<0.01; *** – p<0.001. VODE – volume of damaged epitheliocytes.*

P-order spermatocytes under these experimental conditions was equal to (25.60±0.15)%. This morphometric parameter with a high statistically significant ($p<0.001$) difference exceeded the similar quantitative morphological index in 11.3 times.

The height of spermatogonia of the testes in the experimental animals affected by the studied drug medium decreased by only 1.1% ($p<0.05$), the diameters of their nuclei did not change significantly, and nuclear-cytoplasmic ratios increased by 2.1% ($p<0.05$). The relative volume of damaged spermatogonia in these experimental conditions statistically significantly ($p<0.001$) increased in 13.8 times and reached (29.80±0.15)%.

Analysis of the obtained data revealed that under the influence of rubomycin hydrochloride the height of testicular spermatids decreased by only 1.2% ($p<0.05$), the diameters of their nuclei increased by 3.6% ($p<0.05$). Nuclear-cytoplasmic ratios in the studied cells statistically significantly ($p<0.001$) increased by 7.4% compared with the similar control morphometric parameter. The relative volume of damaged spermatids under these experimental conditions reached (32.50±0.18) %. This morphometric parameter with a high statistically significant difference ($p<0.001$) exceeded the similar control value in almost 15.8 times.

Discussion

Analysis of the above morphometric parameters proved that the introduction of rubomycin hydrochloride into the body of experimental animals led to structural rearrangement and damage to cells of spermatogenic epithelium of the testes. The most pronounced changes were found in spermatids in comparison with other studied cells.

According to present researchers, nuclear-cytoplasmic relations are a valuable morphometric indicator that adequately reflects the peculiarities of cell function and metabolism, degree of disturbances of cellular structural homeostasis [1, 6, 16]. The nucleus and cytoplasm of cells are separated from each other, but at the same time they are closely interconnected and integrated and form a single structural and functional system. It follows that the isolated study of quantitative morphological parameters of only the cytoplasm of the cell or only its nucleus allows getting a one-sided idea of these structural components of the cell. The study of nuclear-cytoplasmic relationships makes it possible to obtain more adequate, objective and in-depth information on the relationship between the nucleus and cytoplasm of cells and their changes in various physiological and pathological conditions. Nuclear-cytoplasmic relations can change at hyperfunction of bodies as well as hypofunction, and also in cases of their damage. It is established that nuclear-cytoplasmic ratios depend on cell differentiation. The specified morphometric parameter decreases in case of maturation of cells and their differentiation that is caused to some extent by increase in cytoplasm and its hyperplasia of specific functioning organelles. The relationship between the spatial characteristics of the nucleus and cytoplasm of the cell can also change with cell division, growth, diploidy [6, 16].

In the optical examination of testicular micro preparations, significant vascular disorders were evidenced, which were characterized by dilation and plethora of mainly venous vessels, which pointed to violation of venous drainage from the studied organs (Fig. 1) [3].

A severe edema of the stroma, foci of dystrophically, necrobiotically altered spermatogenic epitheliocytes, stromal structures, local cellular infiltrates, sclerotic processes, desquamation and proliferation of vascular endothelial cells was present (Pic. 2). The latter pointed to the presence of hypoxia. Foci of vascular endothelial cell edema, permeation of their membrane with plasma proteins were also present. Foci of fibrinoid swelling and necrosis in some vessels were evidenced that indicated their severe damage. Among spermatogenic epitheliocytes in the studied conditions of the experiment, spermatids were the most morphologically altered, in which the degree of disorders

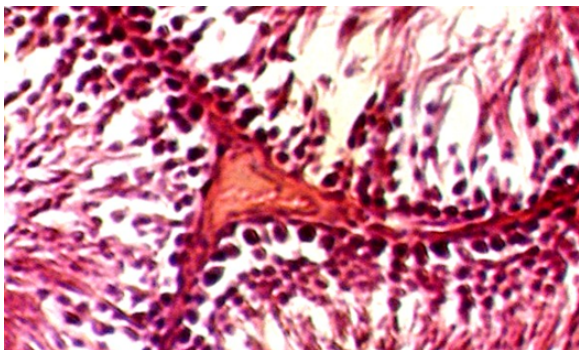

Fig. 1. Stromal edema, partial reduction of layers and damage of spermatogenic epithelial cells, their desquamation, thickening of the membranes of the tortuous seminal tubules of the testis of a white rat under the influence of rubomycin hydrochloride. Hematoxylin-eosin staining. ×140.

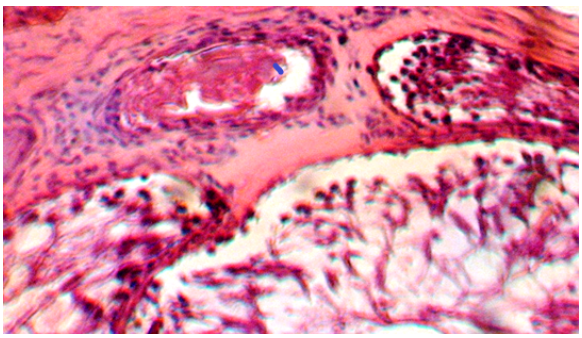

Fig. 2. Structural changes in the vessel wall, perivasal sclerosis, dystrophy, edema, desquamation of spermatogenic epitheliocytes in the tortuous seminal tubules of the testis of experimental animals under the influence of rubomycin hydrochloride. Hematoxylin-eosin staining. ×140.

of nuclear-cytoplasmic relations and the relative volume of damaged cells were the largest. It points to the fact that the studied morphometric parameter also reflects the severity and depth of cell damage.

Conclusions

Nuclear-cytoplasmic relations are an objective and significant informative indicator of functional state of the cells and their structural changes in pathological conditions. When rubomycin hydrochloride is exposed to experimental animals, the most significant changes in nuclear-cytoplasmic relations are found in spermatic spermatozoa.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Hnatiuk M.S. – supervision, morphometry, morphological analysis of micropreparations, writing – review and editing; *Konovalenko S.O.* – writing, generalization of results, project administration, *Tatarchuk L.V.* – supervision, review and editing , *Yasinovsky O.B.* – literature analysis, data curation.

МОРФОМЕТРИЧНА ОЦІНКА СТРУКТУРНОЇ ПЕРЕБУДОВИ КЛІТИН СПЕРМАТОГЕННИХ ЕПІТЕЛІОЦИТІВ СІМ'ЯНИКІВ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН ПІД ВПЛИВОМ РУБОМІЦИНУ ГІДРОХЛОРИДУ

М.С. Гнатюк, С.О. Коноваленко, Л.В. Татарчук, О.Б. Ясіновський

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

Вступ. *За останні 20 років якість сперми чоловіків у всьому світі погіршилась у 2 рази. Причини: погана екологія, стреси, малорухливий спосіб життя, поширеність статевих інфекцій, ожиріння, зловживання алкоголем і різними стимуляторами. Структура та функція сім'яників при дії на організм різних медикаментозних середників та хімічних речовин привертає увагу сучасних дослідників.*

Мета. *Вивчити особливості структурних змін сперматогенного епітелію при ушкодженні сім'яників рубоміцином гідрохлориду.*

Методи. *Морфометрично досліджено сперматогенний епітелій сім'яників 30 білих статевозрілих білих щурів-самців, які були були поділені на 2-і групи. 1-а група включала 15 дослідних інтактних тварин, 2-а – 15 щурів, у яких моделювали ушкодження сім'яників рубоміцином гідрохлоридом. Евтаназію щурів здійснювали кровопусканням в умовах тіопенталового наркозу через місяць від початку досліду. Кількісні морфологічні показники обробляли статистично. У сперматоцитах 1-го. П-го порядків, сперматогоніях та сперматидах сім'яників вимірювали їх висоту, діаметр ядер, ядерно-цитоплазматичні відношення та відносні об'єми пошкоджених клітин сперматогенного епітелію.*

Результати. *Встановлено, що у змодельованих експериментальних умовах пошкоджувалися клітини сперматогенного епітелію, що морфометрично підтверджувалося змінами ядерно-цитоплазматичних відношень у сперматоцитах 1-го, 2-го порядків, сперматогоніях та сперматидах. Виражено зростали також відносні об'єми пошкоджених вказаних клітин сперматогенного епітелію. Найбільш виражено морфометричні параметри виявилися зміненими у сперматидах. Так, ядерно-цитоплазматичні відношення у досліджуваних клітинах статистично достовірно ($p<0,001$) збільшилися на 7,4 % порівняно з аналогічним контрольним морфометричним параметром. Відносний об'єм пошкоджених сперматид у даних умовах експерименту досягав (32,50±0,18) %, що з вираженою статистично достовірною різницею ($p<0,001$) перевищував аналогічну контрольну величину майже у 15,8 рази. Світлооптично на гістологічних препаратах сім'яників спостерігалися виражені судинні розлади, що характеризувалися*

розширенням та повнокров'ям переважно венозних судин, що свідчило про порушення венозного дренажу від досліджуваних органів.

Висновки. *На основі проведених досліджень та отриманих результатів встановлено, що ядерно-цитоплазматичні відношення є об'єктивним та цінним інформативним показником функціонального стану клітин та їх структурних змін в патологічних умовах. При дії на організм дослідних тварин рубоміцину гідрохлориду найвираженіші зміни ядерно-цитоплазматичних відношень виявлені у сперматидах сім'яників.*

КЛЮЧОВІ СЛОВА: **рубоміцин гідрохлорид, сім'яники, сперматогенний епітелій, білі щурі, морфометрія.**

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INFLAMMATION AND IMPACT OF VINCRISTINE AND ENTEROSORPTION USE IN CHEMICALLY INDUCED COLON CARCER IN RATS

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Background. *The increasing incidence of colon malignant tumors is one of the most urgent matters of contemporary medicine. In the study of carcinogenesis of the colon the attention is paid to the state of the body's immune system and activation of inflammatory processes in experimental animals.*

Objective. *The aim of the study was to estimate the level of markers of inflammation in the serum of experimental animals with chemically induced carcinogenesis and their dynamics in case of administration of the cytostatic Vincristine secondary to AUT-M carbon enterosorbent.*

Methods. *The study was performed on white male rats. Animals were modeled for colon cancer by administration of 1.2-dimethylhydrazine hydrochloride at a dose of 7.2 mg/kg body weight for 30 weeks. AUT-M enterosorbent was administered intragastrically daily during 7 and 21 days after modeling of carcinogenesis at a dose of 1 ml of suspension (corresponding to 0.2 g of drug weight) per 100 g of animal body weight. The antitumor drug was administered to the animals with induced carcinogenesis intragastrically daily during 14 days at a dose of 0.23 mg/kg of body weight after a 21-day detoxification therapy. The activity of inflammatory processes was evaluated by the content of pro-inflammatory interleukin 6 and anti-inflammatory interleukin 4, C-reactive protein in the serum of experimental animals.*

Results. *It was established that introduction of 1.2-dimethylhydrazine hydrochloride in the rats caused changes in the cytokine profile and the content of C-reactive protein. In the affected animals an increase in the content of pro-inflammatory interleukin 6, C-reactive protein, as well as a decrease in the content of anti-inflammatory interleukin 4 was evidenced in all periods of the study. AUT-M enterosorbent contributed to normalization of these parameters. The cytostatic Vincristine had a negligible effect on development of inflammatory processes in the studied animals.*

Conclusions. *In cases of induced carcinogenesis, an imbalance in the content of pro- and anti-inflammatory cytokines, an increase in the content of acute-phase C-reactive protein was established. The positive effect of the cytostatic Vincristine secondary to a previous detoxification therapy with AUT-M sorbent during a progressive development of inflammatory processes in the presence of modeled carcinogenesis was evidenced.*

KEY WORDS: **inflammatory processes; proinflammatory interleukins; anti-inflammatory interleukins; AUT-M sorbent; cytostatic Vincristine.**

Introduction

Malignant neoplasms of the colon are still one of the urgent matters of contemporary medicine. Recently the incidence of colorectal cancer among young and middle-aged people has been increasing constantly [1].

It is established that the transformation of a normal cell into a malignant one is accompanied by a homeostasis disorders and changes in all types of metabolism. In the body of the tumour carrier, free radical processes activate, the integrity of plasma membranes is disturbed in cases of destructive changes. As the cancer develops, excessive toxins accumulate and endogenous intoxication intensifies. All these factors cause evolution of inflammatory reaction of the body [2, 3].

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Markers of the systemic inflammatory response in the evolution of carcinogenesis are the acute proteins: ceruloplasmin (CP), haptoglobin and especially C-reactive protein (CRP), cytokines.

C-reactive protein (CRP) is one of the most sensitive and early markers of the acute phase of the inflammatory process. An increase in the concentration of CRP in the serum evidences development of a pathological process and destructive changes in the body [4]. The synthesis of CRP of the acute phase of inflammation is triggered and regulated primarily by cytokines, namely IL-6, which is formed directly in the inflammatory focus and acts as a primary activator of genes involved in it and tumor transformation [5].

Due to the increasing frequency and prevalence of colorectal cancer in modern society, the issue of finding and researching effective

and low-toxic cytostatics of plant origin is still topical.

Vincristine is a herbal cytostatic, it is an important component of the combined treatment regimen for malignant neoplasms. However, in the present literature there are some publications on the use of periwinkle alkaloids in the chemotherapeutic treatment of colorectal cancer [6].

Enterosorption therapy is one of the promising methods for control of inflammation. AUT-M carbon sorbent with a large specific pore area exhibits physicochemical properties of a sorbent substance capable of binding and excreting toxins of exogenous and endogenous origin [7, 8].

The research is aimed to evaluate the level of inflammation markers in the serum of experimental animals with chemically induced carcinogenesis and their dynamics with introduction of the cytostatic Vincristine secondary to AUT-M carbon enterosorbent.

Methods

The study was performed on 76 white male rats. The initial body weight of animals was 200-250 g. Simulation of carcinogenesis of the colon was carried out by subcutaneous weekly injection into the interscapular area of asymmetric 1.2-dimethylhydrazine (1.2-DMG, made by SIGMA-ALDRICH CHEMIE company, Japan) for 30 weeks. The carcinogen 1.2-DMG was previously diluted with isotonic sodium chloride solution, dose 7.2 mg/kg [9].

After modeling of the cancer process for 30 weeks, the affected animals underwent enterosorption therapy for 21 days. AUT-M carbon sorbent was administered intragastrally daily, daily dose 1 ml of suspension (corresponding to 0.2 g of net weight of the drug) per 100 g of body weight of the animal [10].

The anticancer drug Vincristine was administered intragastrically daily at a dose of 0.23 mg/kg of animal body weight for 14 days, starting immediately after 7-month modeling of the cancer process and 21-day enterosorption therapy. The dose of cytostatics was chosen according to the instructions for use of the drug and taking into account sensitivity of the animals (recalculation was carried out according to Yu. R. Rybolovlev, 1979) [11].

The experimental animals were divided into 4 groups: group 1 – the control involved the animals administered with isotonic sodium chloride solution, group 2 – the animals affected by 1.2-DMG, group 3 – the animals affected by 1.2-DMG, managed by AUT-M enterosorbent for 21 days after a 30-week carcinogen administration, group 4 – the animals administered with the cytostatic Vincristine for 14 days after enterosorption therapy.

Euthanasia of animals was performed by bloodletting under thiopental-sodium anesthesia monthly for 7 months and on the 14th and 21st days of administration of the AUT-M enterosorbent after carcinogenesis modeling, as well as on the 14th day after administration of the cytostatic Vincristine. The serum was used for studies.

All manipulations with experimental rats were performed following the rules in accordance with the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes [12].

To study the development of inflammatory processes in cases of induced carcinogenesis of the colon the concentration of proinflammatory and anti-inflammatory interleukins in the serum of rats was determined by the ELISA test [13]. The content of C-reactive proteins was determined by immunoturbidimetric method [14].

The obtained experimental results were statistically processed by the method of variation statistics using a standard package of Statistica 6.0 statistical program [15]. The standard deviations, variance and other statistical parameters were determined, and the statistically significant differences were determined using Student's t-test. The differences were statistically significant at $p \leq 0.05$.

Results

Inflammatory reactions of the body are a non-specific part of the defense, the formation of which prevents the spread of the pathogen and is realized by attracting granulocytes, macrophages and lymphocytes to the site of inflammation. The first stage of such reactions is associated with the activation of pro-inflammatory cytokines. They provide chemotraction of the inflammatory cell [16].

In the dynamics of 1.2-DMG administration, the content of pro-inflammatory cytokine IL-6 in the serum of affected animals probably ($p \leq 0.05$) increased during the 1st month of 1.2-DMG administration (by 62% compare to the control group). Intermediate results point to similar changes: in 5 months of DMG affection (increased by 182%) and in 7 months (by 251%) of adenocarcinoma modeling (Fig. 1).

The corrective dose of the AUT-M sorbent helped to reduce the content of IL-6 in the

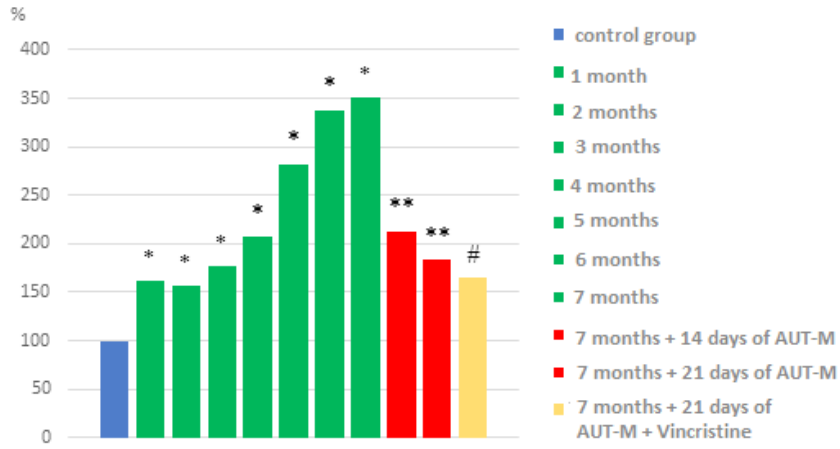

Fig. 1. The level of IL-6 in the serum of rats in the dynamics of the affection by 1.2-DMG and after administration of AUT-M sorbent and cytostatic Vincristine, %.
*Notes: * – statistically significant difference between the parameters of the control group animals and the animals affected by 1.2-DMG; ** – statistically significant difference between the parameters of the carcinogenic animals and the animals administered with enterosorbent; *** – statistically significant difference between the parameters of carcinogenic animals after enterosorption therapy (for 21 days) and the animals administered with cytostatics (for 14 days); # – statistically significant difference between the parameters of carcinogenic animals (in 7 months) and the animals administered with cytostatic (for 14 days) secondary to enterosorption therapy (for 21 days).*

serum of the affected animals. It was established that IL-6 probably decreased in the serum by 138% ($p \leq 0.05$) after a 14-day sorbent therapy, the maximal changes were evidenced in 21 days of sorbent management (decreased by 168%) compare to the same group of the affected animals.

Cytostatic correction performed for 14 days leads to a slight decrease in IL-6 in the serum of rats. The studied parameter decreased by 186% in comparison with the affected animals.

Toxic affection of the animals during 30 weeks causes an imbalance of the cytokine profile in their body. The functional antagonists of pro-inflammatory IL-6 include anti-inflammatory IL-4. This cytokine has an antitumor effect; it is a strong growth factor for B-lymphocytes, which promotes their differentiation, activation and reproduction [17].

Statistically significant differences in the content of IL-4 were found later after beginning of 1.2-DMG administration. The rate decreased by 29% in 5 months of carcinogenesis modeling. After a 30-week DMG affection, the content of IL-4 in the serum of the rats decreased by 45% compare to the control (Fig. 2).

After a 21-day detoxification therapy, the level of IL-4 increased by 30% compare to the group of animals with no detoxification therapy.

In the group of animals undergoing cytostatic therapy, an increase in the content of anti-inflammatory IL-4 by 35% was evidenced compare to the animals with carcinogenesis.

With administration of 1.2-DMG into the experimental animals, changes of acute phase proteins activity were evidenced. Thus, the content of CRP was likely to increase starting the third month of carcinogenesis modeling and exceeded the level of control in 1.5 times. A similar trend was proved in the subsequent affections, in 5 months after the beginning of the experiment, the content of CRP increased in 1.73 times, in 7 months – in 2.1 times compare to the control rats (Table 1).

The obtained results may indicate an intensification of the inflammatory process in the body of experimental animals affected by 1.2 DMG.

The detoxification therapy (for 21 days with the AUT-M sorbent) contributes to the probable decrease on this parameter in the serum of the affected animals. In 21 days of administration of this sorbent, the content of CRP decreased in 1.6 times compare to the group of affected animals.

During the chemotherapy treatment of the affected animals (with previous sorbent correction), a slight increase in the CRP content was evidenced. In 14 days of Vincristine administration, the parameter increased in 1.3 times compare to the animals modeled for colon carcinogenesis for 30 weeks.

Thus, a 30-week DMG affection of the rats caused an imbalance in the content of pro-inflammatory and anti-inflammatory cytokines with further development of inflammatory

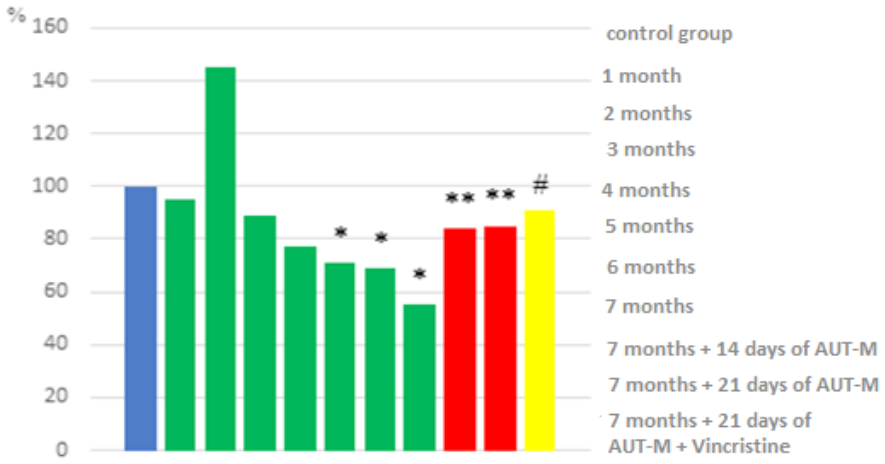

Fig. 2. The content of IL-4 in the serum of rats in the dynamics of the affection by 1.2-DMG and after administration of AUT-M sorbent and cytostatic Vincristine, %

*Notes: * – statistically significant difference between the parameters of the control group animals and the animals affected by 1.2-DMG; ** – statistically significant difference between the parameters of the carcinogenic animals and the animals administered with enterosorbent; *** – statistically significant difference between the parameters of the carcinogenic animals after enterosorption therapy (for 21 days) and the animals administered with cytostatics (for 14 days); # – statistically significant difference between the parameters of the carcinogenic animals (in 7 months) and the animals administered with cytostatics (for 14 days) secondary to enterosorption therapy (for 21 days).*

processes that was confirmed by an increase in early marker of inflammation in the serum: C-reactive protein and interleukin 6. The use of sorption therapy had a positive effect on inflammation in the body of rats throughout the experiment. No significant side effects after administration of the cytostatic Vincristine were evidenced.

Discussion

The search for effective methods of correction of oncological pathologies is topical issue of contemporary medicine. The use of low-toxic and affordable drugs is important. Our research on the efficacy of cytostatic Vincristine secondary to enterosorption therapy in cases of simulated carcinogenesis is proved by reduced

Table 1. The content of C-reactive protein in the serum of rats (mg/l) with induced oncogenesis and after administration of cytostatic Vincristine secondary to detoxification therapy, (M±m)

Animal group/duration of dimethylhydrazine affection	Serum
Control, (n=6)	2.45±0.18
1 month, (n=7)	2.78±0.21
2 months, (n=7)	3.15±0.29
3 months, (n=7)	3.66±0.34*
4 months, (n=7)	4.12±0.38*
5 months, (n=7)	4.25±0.39*
6 months, (n=7)	5.10±0.49*
7 months, (n=7)	5.25±0.50*
7 months of DMG + AUT-M (for 14 days), (n=7)	4.55±0.40
7 months of DMG + AUT-M (for 21 days), (n=7)	3.80±0.36**
7 months of DMG + AUT-M (for 21 days) + Vincristine (for 14 days), (n=7)	4.01±0.16#

*Notes: * – statistically significant difference between the parameters of the control group animals and the animals affected by 1.2-DMG; ** – statistically significant difference between the parameters of the carcinogenic animals and the animals administered with enterosorbent; *** – statistically significant difference between the parameters of the carcinogenic animals after enterosorption therapy (for 21 days) and the animals administered with cytostatic (for 14 days); # – statistically significant difference between the parameters of the carcinogenic animals (in 7 months) and the animals administered with cytostatic (for 14 days) secondary to enterosorption therapy (for 21 days).*

inflammatory manifestations and control of the studied parameters.

In cases of induced pathological process, a violation of the cytokine profile is observed, as the content of the pro-inflammatory cytokine IL-6 increases, the content of the anti-inflammatory cytokine IL-4 decreases.

The obtained results point to an imbalance of the cytokine profile with an advantage towards IL-6. It is established that IL-6 is one of the main inducers of the acute phase response in inflammatory process. Obviously, these changes may evidence of development of chronic inflammation and tumor progression [18].

The system of immunoregulation in the body of the tumor carrier along with the cytokine cascade is followed by changes in the content of CRP. In cases of cytokine profile imbalance in the affected animals, an increase in the CRP content was evidenced. It is established that CRP is an acute marker of inflammation, so the results point towards development and progression of inflammatory processes [19].

Therefore, it has been established that the affection of the experimental animals with 1.2-DMG for 7 months leads to an imbalance of the cytokine profile, an increase in the content of acute phase CRP proteins. No significant side effects of the cytostatic Vincristine are evidenced that proves a positive effect of detoxification therapy with the AUR sorbent during the progressive inflammatory process in cases of modeled oncological process.

Conclusions

A long-term increase in the content of pro-inflammatory interleukin 6 in addition to reduced production of anti-inflammatory interleukin 4, as well as an increase in C-reactive protein in the rats affected with 1.2-dimethylhydrazine hydrochloride for 30 weeks that evidences development of inflammatory processes in the carcinogenesis body.

After detoxification therapy with AUT-M carbon sorbent a decrease in inflammatory processes activity in the animals with experimental carcinogenesis was present. This is proved by a decrease in the content of pro-inflammatory interleukin 6, C-reactive protein and an increase in the content of anti-inflammatory interleukin 4.

The positive effect of Vincristine, a cytostatic drug of plant origin, on the activity of inflammatory processes in the rats in cases of experimental carcinogenesis has been established. It has been experimentally proved that this cytostatic has no side effects obviously as a result of previous enterosorption therapy.

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Conflict of Interest

The author declares no conflict of interest.

Author Contributions

Kachur O.I. – resources, investigation, formal analysis, writing – original draft; *Fira L.S.* – conceptualization, formal analysis, writing – review & editing; *Lykhatskyi P.H.* – conceptualization, project administration, supervision.

РОЗВИТОК ЗАПАЛЬНИХ ПРОЦЕСІВ У ЩУРІВ ІЗ ІНДУКОВАНИМ КАНЦЕРОГЕНЕЗОМ ТОВСТОЇ КИШКИ ПІСЛЯ ЗАСТОСУВАННЯ ЦИТОСТАТИКУ ВІНКРИСТИН НА ТЛІ ЕНТЕРОСОРБЦІЇ

О.І. Качур, Л.С.Фіра, П.Г.Лихацький

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

Вступ. *Проблема зростання частоти розвитку злоякісних пухлин товстої кишки – одна з найбільш актуальних у медицини. Об'єктом уваги при вивченні канцерогенезу товстої кишки є стан імунної системи організму та активація запальних процесів в експериментальних тварин. Перспективним є проведення хіміотерапії рослинними препаратами на тлі ентеросорційної корекції.*

Мета дослідження – *оцінити рівень маркерів запалення в сироватці крові експериментальних тварин із хімічно індукованим канцерогенезом та їх динаміку при введенні цитостатика Вінкристин на тлі вуглецевого ентеросорбенту АУТ-М.*

Методи дослідження. *Дослідження проводили на білих щурахсамцях. Тваринам моделювали рак товстої кишки шляхом введення 1,2диметилгідразин гідрохлориду в дозі 7,2 мг/кг маси тіла протягом 30ти тижнів. Ентеросорбент АУТ-М вводили інтрагастрально щоденно впродовж 21ї доби після*

моделювання канцерогенезу в дозі 1 мл завису (що відповідає 0,2 г чистої маси препарату) на 100 г маси тіла тварини. Протипухлинний препарат вводили тваринам з індукованим канцерогенезом внутрішньошлунково щоденно протягом 14-ти днів у дозі 0,23 мг/кг маси тіла після 21-денної детоксикаційної терапії. Активність запальних процесів оцінювали за вмістом прозапального інтерлейкіну 6 та протизапального інтерлейкіну 4, Среактивного протеїну в сироватці крові експериментальних тварин.

Результати. *Встановлено, що введення щурам 1,2диметилгідразин гідрохлориду супроводжується зміною цитокінового профілю та вмісту Среактивного протеїну. В уражених тварин спостерігали збільшення вмісту прозапального інтерлейкіну 6, Среактивного протеїну, а також зменшення вмісту протизапального інтерлейкіну 4 в усі терміни дослідження. Застосування ентеросорбенту АУТ сприяло нормалізації цих показників. Введення цитостатика Вінкристин незначно вплинуло на розвиток запальних процесів у досліджуваних тварин.*

Висновки. *За умов індукованого канцерогенезу встановлено дисбаланс у вмісті про- та протизапальних цитокінів, збільшення вмісту гострофазового С-реактивного протеїну. Відмічено позитивний вплив цитостатика Вінкристин на тлі попередньо проведеної детоксикаційної терапії сорбентом АУТ під час прогресуючого розвитку запальних процесів за умов змодельованого канцерогенезу.*

КЛЮЧОВІ СЛОВА: **запальні процеси; прозапальні інтерлейкіни; протизапальні інтерлейкіни; сорбент АУТ-М; цитостатик Вінкристин.**

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WEB-BASED 5-DIMENSIONAL ELECTRONIC PORTFOLIO (5DEP) AS A COMPETENCY-BASED ASSESSMENT TOOL IN POST-GRADUATE MEDICAL TRAINING

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Background. *New challenges of permanently changing context of healthcare system requires new methods of medical education and new assessment tools, as well. Competency-based Medical Education (CBME), framework which has been adopted as a new approach in medical education, needs appropriate assessment tool such as portfolio. Portfolio is learner-centered assessment instrument which evaluates learner's progression towards outcomes and enables both residents and teachers to engage in a process of learning through assessment.*

Objective. *In this paper we aim to share our successful experience of an effective use of web-based 5-Dimensional Electronic Portfolio (5DeP) as an assessment tool in the Pilot Group.*

Methods. *Pilot Group of sixteen residents (six first year residents of Obstetrics/Gynecology and ten first year residents of Internal Medicine. Tbilisi State Medical University Institute of Postgraduate Medical Education and Continuous Professional Development) and twelve mentors (four Obstetrics/Gynecology mentor and eight Internal Medicine mentors) reported some feedback about 5-Dimensional Electronic Portfolio (5DeP) as a new assessment tool.*

Results. *Feedback about 5-Dimensional Electronic Portfolio (5DeP) as a new assessment tool from mentors and students demonstrated efficiency of the program. It enables assessment within a framework of transparent and declared criteria and learning objectives; provides a model for lifelong learning and continuing professional development; increases competence in a wider context with benefits to both professional and personal roles; improves organizing skills.*

Conclusions. *5DeP have been recognized as an extremely effective assessment tool.*

KEY WORDS: **Competency-based Medical Education (CBME); postgraduate education; electronic portfolio; feedback.**

Introduction

The fundamental changes in health care and the complexity of health problems have radically changed the role of Physicians in the health care system and mounted different challenges in terms of their under- and post-graduate education, and continuous professional development.

Over the last two decades, Competency-Based Medical Education (CBME) frameworks have been internationally adopted as the primary educational approach in medicine. CBME is a learner-centered, active, and lifelong experience that incorporates feedback between the teacher and the learner to fulfill the desired competency outcomes [1].

Despite standardized core competencies of medical education, there are no standardized assessment methods to determine learner's achievement in all the core competencies prior to completion of residency training [1]. Competency-based education needs continuous, comprehensive feedback and assessment systems [2]. The use of one of most popular assessment instruments, portfolio can facilitate the above-mentioned goals of assessment by collecting information about the learner's progression towards outcomes [3]. Portfolio-based assessment tool is at the apex of Miller's pyramid, because it provides performance-based assessment in real context by analysis of actions [4, 5].

Smith and Tillema made four different types of portfolios [6]:

1. The dossier portfolio, containing mandated records of achievement.

2. The training portfolio, containing a mandated collection of acquired skills and competencies, in a fixed format, with some reflective comments on selected evidence.

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3. The reflective portfolio, containing a purposeful collection of evidence for personal growth and development, and

4. The personal development portfolio, containing reflective and personal evaluations of progress in time, enabling discussion and valuing of these activities.

In this paper, we aim to provide guidance to program directors for implementation of an electronic portfolio (e-portfolio) by reporting our successful experience of an effective use of web-based e-portfolio system in Internal Medicine and Obstetrics/Gynecology residency programs of Tbilisi State Medical University, where the 5-Dimensional Electronic Portfolio (5DeP) was introduced with support of United Nations Population Fund (UNFPA) in 2015.

Methods

The Pilot Group of sixteen residents (six first year residents of Obstetrics/Gynecology and ten first year residents of Internal Medicine. Tbilisi State Medical University Institute of Postgraduate Medical Education and Continuous Professional Development) and twelve mentors (four Obstetrics/Gynecology mentor and eight Internal Medicine mentors) reported some feedback about 5-Dimensional Electronic Portfolio (5DeP) as a new assessment tool.

Design and structure of 5-Dimensional Electronic Portfolio (5DeP)

The new web-based 5-Dimensional Electronic Portfolio (5DeP) is designed in simple and intuitive manner for users with minimal experience and skills with information technologies. The three modules with different levels of access by individual username and password are designed for (Fig.1):

• Resident (Module 1, access level A), to:

– upload/confirm evidences of performance/achievements during training course, approved by the Mentors

– view of Training Course Assessment Form (TCAF) and

– view of Final Portfolio Assessment Form (FPAF) and narratives

• Mentor (Module 2, access level B), for resident training place assessment (TPAF);

• Program Director/Experts Panel (Module 3, access level C), for monitor of training process and final assessment of portfolio (IPAF/FPAF).

Module 1 – Collected Evidences (CE)

This module covers all the four types of Smith's & Tillema's stratification [6] and is intended for residents to:

• upload evidences collected during all training courses:

– achievement of learning outcomes;

– clinical (inpatients/outpatients) cases, write-ups and shift records;

– acquired essential skills;

– self-evaluation of progress in time.

• view Training Course Assessment Form (see below) after completion each training course;

• self-monitor of dynamics on each dimension during the residency training process.

Module 2 – Training Course Assessment Form (TCAF)

This module is intended for mentors (clinical trainers) to assess resident on following five dimensions at the end of each training course:

• **Communication** – by evaluation of a resident's interpersonal communication skills, ability to work as integral part of a multidisciplinary medical team, to prevent and manage of communication barriers. This dimension also looks at the strengths of resident's medical record keeping skills;

• **Self-development** – by evaluation of resident's attitude and responsibility for his/her own personal development, ability to take active part in learning opportunities, and effective use of reflection; This dimension also looks at the resident's ability to follow patient/staff/self-safety and security policy;

• **Professionalism** – by evaluation of resident's attitude to work, professional appearance, timekeeping, professional boundaries, quality assurance; ability to maintain patient confidentiality, and recognize and respect people's diversity, preferences, choices and beliefs;

• **Assessment and planning** – by evaluation resident's ability to use basic knowledge and analytical skills in patient assessment and clinical reasoning, collaborate with patient in decision making process and elaborate appropriate management plan;

• **Intervention and treatment** – by evaluation resident's ability to identify specific precautions/contraindications to the intervention/treatment, deliver intervention/treatment with skill and care, and take appropriate actions in case of complications.

Each dimension contains marking guide table with detailed graded descriptors. Choosing specific descriptor that mostly accurately describes the resident's performance (Excellent: 5 points; Very good: 4 points; Good: 3 points;

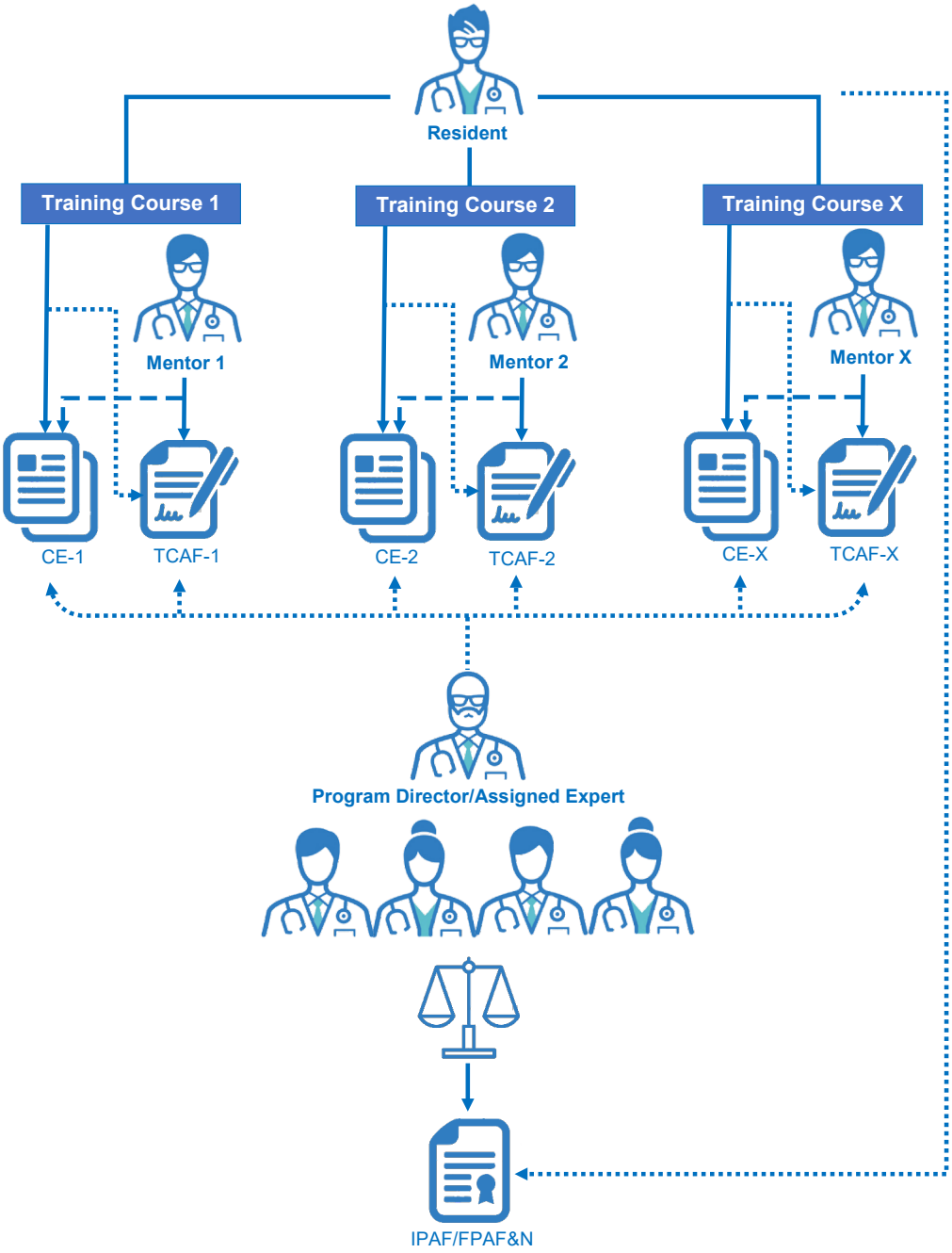

Figure 1. 5-Dimensional Electronic Portfolio (5DeP). Design and structure.
CE: Collected evidences (form training courses 1, 2 and X). TCAF: Training Course Assessment Form (made by the Tutor 1, 2 and X of training courses 1,2 and X, respectively); FPAF&N: Final Portfolio Assessment Form and Narratives. Solid line: to upload/edit/confirm; Dotted line: to view; Long dashed line: to approve/confirm.

Average: 2 points; Below average: 1 point) the module calculates final score for specific dimension and turns the score into an appropriate grade with percentage mark (A –Excellent: 91-100% of the maximum grade; B – Very good: 81-90% of the maximum grade; C – Good: 71-80% of the maximum grade; D – Satisfactory: 61-70% of the maximum grade; E – Sufficient: 51-60% of the maximum grade; Fx – Fail: 41-50% of the maximum grade and F – Fail: >50% of the maximum grade).

At the end of the training course the mentor should complete and approve (without further permission of editing) the Training Course Assessment Form (TCAF) with the Net Diagram (D-Net) for marks and grades visualization

(Fig. 2). After completion of the training course mentor will meet with the resident to discuss his/her overall performance. During the meeting should be discussed in which areas the resident has been deficient and why.

Module 3 – Interim Portfolio Assessment Form/Final Portfolio Assessment Form (IPAF/FPAF)

This module is designed for the Program Director to perform consistent monitoring of training process and provide:

• interim portfolio assessment at the end of each residency year

• final portfolio assessment at the end of residency course chairing the Experts Panel

• report with analysis of resident's performance and progression towards outcomes during the training courses and final D-Net.

Results

The Pilot Group of sixteen residents (six first year residents of Obstetrics/Gynecology and ten first year residents of Internal Medicine. Tbilisi State Medical University Institute of Postgraduate Medical Education and Continuous Professional Development) and twelve mentors (four Obstetrics/Gynecology mentor and eight Internal Medicine mentors) reported some feedback about 5-Dimensional Electronic Portfolio (5DeP) as a new assessment tool. The mentors found that 5DeP:

• is customized to meet the needs of the residency programs;

• is easy to understand and efficient to use;

• deals with the inherent deficiencies of traditional system of evaluation;

• enables assessment within a framework of transparent and declared criteria and learning objectives;

• evaluates and stimulates progress towards educational and professional outcomes;

• evaluates learning outcomes not easily assessed by other methods;

• provides summative assessment of progress and formative evaluation;

• improves mentor awareness of student's need and support required for students;

• enhances of interactions between residents and mentors;

• focusses on resident's personal attributes;

• motivates mentors to focus on the training objectives;

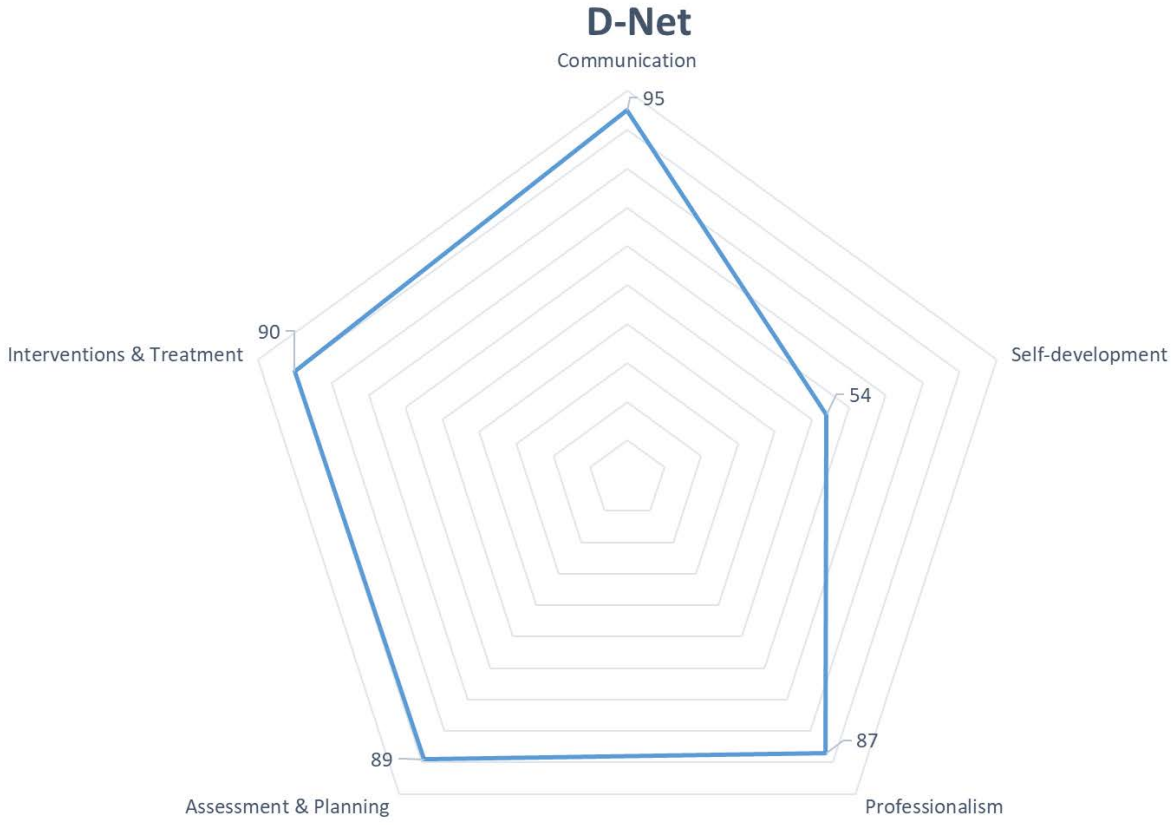

Figure 2. Example of D-Net.
The Resident's excellent performance in all dimensions is visualized by D-Net, except "Self-development".

• can accommodate evidence of learning from a range of different contexts;

• allows a range of learning styles to be used according to the preferences of the student;

• provides a model for lifelong learning and continuing professional development.

The students found that 5DeP:

• allows to understand learning objectives, using specific targets;

• enables the consolidation of the connection between theory and practice, and apply abstract principles to practical contexts;

• recognizes and encourages the autonomous and reflective learning that is an integral part of professional education and development;

• enhances self-learning and self-development;

• enables to correct errors and remedies deficiencies;

• focusses on resident's personal attributes;

• evaluates and stimulates progress towards educational and professional outcomes;

• enhances of interactions between residents and mentors;

• motivates both residents to focus on the training objectives;

• helps to update of knowledge and skills in existing and new areas of practice;

• increases competence in a wider context with benefits to both professional and personal roles;

• improves organizing skills;

• helps to estimate potential to progress to other levels or courses.

Discussion

The use of portfolio as an assessment instrument enables residents and teachers to engage in a process of learning through assessment. The use of portfolio broadens the scope of assessment and introduces several educational benefits [7, 8, 9, 10]:

• Contribution to:

– the assessment of learning outcomes

– the provision of evidence of performance

– the representation of evidence collected over a period

– resident progression towards the learning outcomes

– summative and formative assessment

• Focus on personal attributes

• Enhancement of interactions between residents and teachers

• Stimulation of the use of reflective strategies

• Encouragement of a holistic and integrative approach to medical practice.

The portfolio assessment remains relatively unknown among clinical teachers and residents [11, 12, 13, 14, 15].

Conclusions

5-Dimensional Electronic Portfolio (5DeP) have been recognized as an extremely effective assessment too that could provide motivation to study, can assist students in forming positive attitudes toward learning, to enable students to individualize and personalize their learning by supporting and encouraging active participation.

Conflict of interest

The authors declare no conflict of interest.

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Author's contribution

Zaza Bokhua – contributed to the data curation, formal analysis, funding acquisition, investigation, resources and writing original draft; *Kakhaber Chelidze* – contributed to the conceptualization, formal analysis, methodology, project administration, supervision, visualization, validation and review and editing of original draft; *Ketevan Ebralidze* – contributed to the formal analysis, and review and editing of original draft.

П'ЯТИВИМІРНЕ ЕЛЕКТРОННЕ ПОРТФОЛІО (5DEP) ЯК ІНСТРУМЕНТ ОЦІНКИ КОМПЕТЕНТНОСТЕЙ У ПІСЛЯДИПЛОМНОМУ МЕДИЧНОМУ НАВЧАННІ

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Вступ. *Сучасні виклики у системі охорони здоров'я, зумовлені постійними її змінами, вимагають новітніх методів навчання та інструментів їх оцінки. Компетентнісний підхід до навчання, котрий було взято за основу сучасних стандартів професійної медичної освіти, потребує відповідних методів оцінки, таких як портфоліо. Портфоліо – це інструмент, сконцентрований на простежуванні прогресу*

набутих знань особою, що навчається, який дозволяє зацікавити і залучити до процесу не лише резидента, а й викладача.

Мета – *поділитися успішним досвідом застосування 5-вимірного електронного портфоліо (5DeP) у якості інструменту оцінки набуття професійних компетентностей у пілотній групі.*

Методи. *Пілотну групу склали 16 резидентів (шість з них – резиденти першого року навчання зі спеціальності Акушерство/гінекологія, ще десять – першого року навчання зі спеціальності Внутрішня медицина Інституту післядипломної медичної освіти та безперервного професійного розвитку, Тбіліський державний медичний університет) та дванадцять менторів-наставників (чотири з акушерства/гінекології та вісім – з Внутрішньої медицини). Учасники пілотного проекту ділилися відгуками щодо оцінки застосування 5-вимірного електронного портфоліо (5DeP) у якості інструменту оцінки набуття професійних компетентностей*

Результати. *Отримані у процесі застосування 5-вимірного електронного портфоліо (5DeP) відгуки та зворотній зв'язок від учасників пілотної групи (менторів та резидентів) продемонстрували ефективність програми як інструменту оцінки набуття професійних компетентностей. Портфоліо забезпечує прозорі та чіткі критерії оцінки знань та компетентностей, забезпечує модель безперервного професійного розвитку на "навчання протягом усього життя", підвищує рівень як професійних так і особистісних компетентностей, покращує організаційні навики.*

Висновки. *5DeP – надзвичайно ефективний інструмент оцінки набутих знань та компетентностей.*

КЛЮЧОВІ СЛОВА: **компетентнісно орієнтована медична освіта (CBME); післядипломна освіта; електронне портфоліо; зворотній зв'язок.**

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