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KIDNEY LESIONS IN HIV-INFECTED PATIENTS

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Introduction. *HIV prevalence is one of the most important issues of contemporary medicine. Over a 30-year history of this disease more than 75 million people have been infected with HIV, nearly 30 million adults and children of died. In the future decades, its significance in world premature mortality rates continues to rise. The objective of the study was to establish clinical and laboratory features of kidney lesions in HIV infection.*

Methods. *The study involved 292 HIV-infected patients, who were managed outpatiently at the Chernivtsi Regional AIDS Center. Taking into account the main markers of kidney lesions: persistent proteinuria and glomerular filtration rate <60 mL/min/1.73 m^2, 48 persons were diagnosed with chronic kidney disease (CKD), which was very frequently accompanied by dysfunction of these organs.*

Results. *Increasing proteinuria rate is accompanied by a significant renal dysfunction and more frequently is combined with arterial hypertension as well as hematuria without significant differences in the incidence of opportunistic diseases. The mean reciprocal correlation between the levels of proteinuria and glomerular filtration rate (r=-0.562, p<0.01), as well as between the levels of proteinuria and hemoglobin (r=-0.596, p<0.01) have been established as well.*

Conclusions. *Kidney lesions in HIV-infected are most often characterized by tubulointerstitial lesions. At the same time, glomerular kidney lesion, which is much less common, is accompanied by a significantly higher level of HIV RNA.*

KEY WORDS: **HIV-infection; chronic kidney disease; tubulo-interstitial lesion; glomerular lesion of kidneys.**

Introduction

HIV prevalence is one of the most important issues of contemporary medicine. Over a 30-year history of this disease more than 75 million people have been infected with HIV, nearly 30 million adults and children died [1, 2]. In the future decades, its significance in world premature mortality rates continues to rise.

The kidneys lesion, which is often characterized by severe clinical manifestations, can significantly affect the life expectancy in HIV-infected patients [3, 4]. Considering the increasing number of HIV-infected people in the world and a rise in the life expectancy of such patients, an increase in the number of HIV-infected people in need of expensive substitution renal therapy as well as kidney transplantation is expected.

The world scientific literature points out the factors associated with renal impairment in HIV infection: history of kidney disease, uncontrolled HIV infection, time spent on HAART, older age, female sex, African origin (APOL1 genetic variant), CD4$^+$ lymphocytes <200 cells/ml, as well as the use of nephrotoxic drugs [5].

However, despite a large number of foreign publications concerning this topic, the issue of the kidney lesion in HIV infection is studied insufficiently in Ukraine.

The objective of the study was to establish clinical and laboratory features of kidney lesions in HIV infection.

Methods

The study involved 292 HIV-infected patients, who were managed outpatiently at the Chernivtsi Regional AIDS Center (Chief Physician V. M. Mochulskyi).

In establishing the diagnosis, clinical and epidemiological data as well as findings of the laboratory examination methods: serological and immunological (including determination of CD4$^+$-lymphocyte contents), were taken into account. The initial screening of HIV-infected people was carried out, when they were registered for monitoring in accordance with the clinical protocol No. 551, dated July 12, 2010.

The average age of all patients was (29.3±8.2) years (ranged from 19 to 55 years). There were 188 (64.4%) men and 104 (35.6%)

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women among the patients. The study mostly involved young patients (25-44 years old). Among the patients, who were included in the study, 26 (8.9%) were diagnosed with the first stage of HIV infection, 40 (13.7%) individuals – with the second one, 108 (37.0%) patients – with the third, and 118 (40.4 %) were diagnosed with the fourth clinical stage of the disease.

The screening of kidney lesion markers with albuminuria/proteinuria test systems by means of urinary strips (Aution Sticks-2EA) was performed. With the presence of proteinuria ≥1+ in the screening test, corresponding to a gradation of 30 mg/l, repeated urinalysis was performed with a quantitative protein determination by means of MIKROLAB-600 spectrophotometer using UNI-TEST-BM reagents separated in the period from 3 days to one week.

The functional status of the kidneys was evaluated by the integral index, which characterized the degree of active nephrons mass maintenance/loss. A decrease in glomerular filtration rate (GFR) <60 mL/min per 1,73 м^2 was a criterion of renal dysfunction [6, 7]. Chronic kidney disease was diagnosed when proteinuria or that in combination with a decrease in GFR for 3 months or more was revealed.

A screening study to identify the kidney lesion markers (permanent proteinuria, reduction in GFR that was detected for 3 or more months) in HIV-infected patients was conducted in accordance with the recommendations of the Kidney Disease Outcome Quality Initiative, K / DOQI, 2002, and Infectious Diseases Society of America, IDSA, 2005 [4, 7]. Among the surveyed patients there were 105 (36.0%) people with markers of kidney lesion: albuminuria/ proteinuria. Based on the main markers of kidney lesion: persistent proteinuria (PU) and GFR <60 mL/min/1.73 m^2, in 16.4% of cases chronic kidney disease (CKD) was diagnosed, which was very frequently accompanied with renal impairment.

HIV-associated nephropathy was revealed in 48 out of 292 (16.4%) patients (31 men and 17 women), in whom the markers of kidney lesion: persistent proteinuria or proteinuria combined with a decrease in GFR, were identified and confirmed in the course of the examination.

Statistical processing of the received data was carried out using the package of applications STATISTICA 6.1 (StatSoft, USA) and Microsoft Excel 2007 programs.

The normal dissemination of the signs was determined by the graphical method, the Lilliefors criterion and the W-criterion of Shapiro-Wildlife. Dispersion of attributes was evaluated using the F-criterion in the ANOVA dispersion analysis procedure. To describe the selective normal distribution of quantitative attributes, the arithmetic mean (M) and standard deviation (m) were calculated. If the dissemination of the sign differed from normal, for its description the median (Me) and the interquartile scale with the boundaries of the segment [25%; 75%] was developed.

When comparing several independent groups, the Crackel-Wallis dispersion analysis was used (to avoid multiple comparisons). Nonparametric methods were used to compare two independent groups: the Mann-Whitney U-test and the Kolmogorov-Smirnov test, and the two dependent groups were for the Wilcoxon criterion.

The correlation analysis of two quantitative attributes was carried out using Spierman's rank method: the relationship between the indicators was considered weak in case $r<0.3$, moderate – at $0.3<r<0.7$, strong – at $r>0.7$.

The comparison of groups by qualitative features was carried out by nonparametric method through analyzing 2×2 conjugation tables using a two-sided exact Fisher or χ^2 for unrelated groups.

Multivariate logistic regression analysis was used to identify the predictors of kidney impairment. Statistical differences were significant at $p<0.05$, very significant at $p<0.01$, the most significant at $p<0.001$ and insignificant at $p>0.05$.

When describing qualitative signs, the percentage of patients with the presence or absence of the analyzed sign from the total number of patients in the group is presented. The results of studies, processed statistically and presented in tables or diagrams, allow establishing the dynamics of the parameter, reliability, as well as the relationship with the changes in other parameters in accordance with existing requirements.

Results

48 HIV-infected patients with kidney lesion had the following distinctive clinical symptoms and syndromes of CKD:

– urinary syndrome characterized by isolated proteinuria of varying degrees, by proteinuria in combination with hematuria/leukocyturia;
– arterial hypertension (AH);
– acute nephritic syndrome;
– nephrotic syndrome;

– chronic renal insufficiency.

It was established that in every fourth HIV-infected person with CKD the urinary syndrome was characterized by isolated PU (27.1%). PU was most often combined with changes in the urine sediment: erythrocyturia and leukocyturia (17 persons – 35.4%) or hematuria (14 patients – 29.2%), with the latter most often accompanied by PU>1.0 g/day compared with the group of patients with a lower level of protein in the urine (90.5 and 51.9% respectively, p<0.01). In 4 patients (8.3%) PU was combined with leukocyturia. It should be noted that in more than half of patients transient non-bacterial leukocyturia was evidenced – more often at PU≤1.0 g/day.

AH was diagnosed in 15 patients (31.3%) in the presence of proteinuria compared with 2.5% in its absence (p<0.001). Acute nephritic syndrome was revealed in 5 patients (10.4%), nephrotic syndrome – in 7 (14.6%), reduction of GFR<60 mL/min/1.73 m^2 – in 23 individuals (47.9%).

According to the analysis of complaints, anamnestic information and clinical symptoms of kidney lesion, the patients were divided into 2 groups. The first group consisted of 31 (64.6%) out of 48 persons with tubulointerstitial and the second one – of 17 (35.4%) patients with glomerular diseases (Table 1). The presented data confirm that HIV-infected kidney lesions are most often characterized by tubulointerstitial lesion.

Chronic tubulointerstitial diseases of kidneys were characterized by a minimal or insignificant PU (0.4 [0.3; 0.8] g/day) and only in 4 (12.9±6.0)% of patients it exceeded 1 g/day. PU only was evidenced in 9 – (29.0±8.1)% of cases, but in most people PU was combined with changes in urine sedimentation. For instance, PU was accompanied by hematuria, manifested by isomorphous erythrocytes and leukocyturia in 8 (25.8±7.9)% of patients, hematuria – in 2 (6.5±4.4)% and leukocyturia – in 4 (12.9±6.0)% of cases.

In tubulointerstitial diseases, in comparison with the glomerular pathology of kidneys, the renal function impairment was diagnosed much less frequently (32.3±8.4) against (76.5±10.3) (p<0.01), as well as AH – (9.7±5.3) and (70.6±11.0)% respectively (p<0.001).

Glomerular kidney lesion was characterized by a significantly lower glomerular filtration rate – 48.7 [30.2; 78.9] vs. 84.5 [52.6; 107.2] mL/

Table 1. Clinical characteristics of patients with different variants of kidney damage

Criterion	Damage to the kidneys		All patients (n=48)
	Tubulointerstitial diseases (n=31)	Glomerular diseases (n=17)	
GFR, mL/min/1.73 m^2, median [25%; 75%]	84.5 [52.6; 107.2]	48.7 [30.2; 78.9]*	60.2 [34.4; 80.3]
≥90, n (M%±m%)	15 (48.4±9.0) %	1 (5.9±5.7) %*	15 (31.3±6.7) %
60-89, n (M%±m%)	8 (25.8±7.9) %	3 (17.6±9.2) %	10 (20.8±5.9) %
30-59, n (M%±m%)	7 (22.6±7.5) %	8 (47.1±12.1) %	16 (33.3±6.8) %
15-29, n (M%±m%)	1 (3.2±3.2) %	2 (17.6±9.2) %	3 (6.3±3.5) %
<15, n (M%±m%)	0 (0.0±0.0) %	3 (17.6±9.2) %	4 (8.3±4.0) %
Renal impairment, n (M%±m%)	10 (32.3±8.4) %	13 (76.5±10.3) %*	22 (45.8±7.2) %
Proteinuria, g/day, median [25%; 75%]	0.4 [0.3; 0.8]	1.3 [1.4; 3.0]*	0.8 [0.34; 1.42]
≤1 g / day, n (M%±m%)	27 (87.1±6.0) %	0 (0.0±0.0) %*	27 (56.3±7.2) %
>1 g / day, n (M%±m%)	4 (12.9±6.0) %	17 (100.0±0.0) %*	21 (43.8±7.2) %
Isolated proteinuria, n (M%±m%)	9 (29.0±8.1) %	3 (17.6±9.2) %	12 (25.0±6.3) %
Proteinuria and hematuria, n (M%±m%)	2 (6.5±4.4) %	11 (64.7±11.6) %*	14 (29.2±6.6) %
Proteinuria, hematuria, leukocyturia, n (M%±m%)	8 (25.8±7.9) %	9 (52.9±12.1) %*	17 (35.4±6.9) %
Proteinuria and leukocyturia, n (M%±m%)	4 (12.9±6.0) %	0 (0.0±0.0) %*	4 (8.3±4.0) %
Acute Nephritis Syndrome, n (M%±m%)	0 (0.0±0.0) %	5 (29.4±11.0) %*	5 (10.4±4.4) %
Nephrotic syndrome, n (M%±m%)	0 (0.0±0.0) %	7 (41.2±11.9) %*	7 (14.6±5.1) %
Arterial hypertension, n (M%±m%)	3 (9.7±5.3) %	12 (70.6±11.0) %*	15 (31.3±6.7) %
Hemoglobin, g/l, median [25%; 75%]	124.0 [112.5; 133.0]	99.1 [83.0; 123.6]*	111.5 [85.5; 131.0]

Notes: * – significant difference between the groups of patients with glomerular and tubulointerstitial diseases (p<0.05-0.001).

min/1.73 m^2 (p<0.05). Accordingly, only 1 person with glomerular lesion had GFR higher than 90 mL/min/1.73 m^2, which was significantly lower than the corresponding frequency of this feature in tubulointerstitial pathology – (48.4±9.0)% (p<0.001). At the same time, the final stage of CKD was in 3 (17.6±9,2)% of patients, 2 of whom were recommended substitution renal therapy by hemodialysis program.

In cases of glomerular kidney lesion there was also a significantly higher level of PU – 1.3 [1.4; 3.0] vs. 0.4 [0.3; 0.8] g/day (p<0.05). For instance, it exceeded 3.0 g/day in 8 patients and reached 8.0 and 9.0 g/day in 2 of them. The combination of PU with hematuria (64.7±11.6) and (6.5±4.4)%, respectively (p<0.001), with hematuria and aseptic leukocyturia (52.9±12.1) and 25.8±7.9)%, respectively (p<0.05) were present much more frequently than in the patients with tubulointerstitial diseases. Thus, in the majority of cases there was microhematuria, manifested by dysmorphic erythrocytes, whereas episodic macrohematuria was evidenced in 2 patients.

7 (41.2±11.9)% patients were diagnosed with nephrotic and 5 (29.4±11.0)% people suffered from acute nephritic syndromes. It is noteworthy, that these syndromes were not revealed in any representative of the group with tubulointerstitial disease. Expectedly, the level of hemoglobin in glomerular kidney lesion was reliably lower: 99.1 [83.0; 123.6] vs. 124.0 [112.5; 133.0] g/l (p<0.05).

This may point to the direct effect of HIV on the glomerular apparatus, whereas tubulointerstitial kidney lesions are most likely due to the influence of opportunistic infections and drugs with nephrotoxic potential, as well as the use of psychotropic drugs and the uncontrolled administration of nonsteroidal anti-inflammatory drugs, which these patients often abuse of.

The mean number of $CD4^+$ lymphocytes in serum of the patients with proteinuria is much lower than in the HIV-infected individuals without markers of kidney lesion: 185.5 [25-60.9; 75% – 318.0] vs. 312.0 [25% – 175.5; 75% – 469.0] cl/μl respectively (p<0.05). A decrease in $CD4^+$ lymphocytes level ≤200 cl/μl was found in 52.1±7.2% of patients with proteinuria and in 30.0±7.2% of those without it (p<0.05). The difference between the ratios of $CD4^+/CD8^+$-lymphocytes in the studied groups was also quite significant: 0.2 [0.1; 0.4] and 0.4 [0.2; 0.6] respectively (p<0.05) (Table 2).

The data concerning the HIV RNA level and type of CKD are presented at Fig. 1.

Depending on the level of proteinuria, the patients were divided into two groups. The first group consisted of 27 out of 48 (56.3%) patients with PU less than 1.0 g/day, the second group – 21 (43.7%) patients with PU more than 1.0 g/day, in 7 of them it reached the nephrotic level – more than 3.0 g/day.

There were more males in both groups (70.4±8.4) and (57.1±10.9)% respectively, and people aged 25-44 (66.7±9.1) and (61.9±10,6)%

Table 2. Number of RNAs of HIV, $CD4^+$ lymphocytes and the ratio of $CD4^+/CD8^+$ in the patients with different variants of clinical kidney damage

Criterion	Damage to the kidneys		All patients (n=48)
	Tubulointerstitial diseases (n=31)	Glomerular diseases (n=17)	
Viral load (RNA of HIV), copies/ml	22 000 [5 125; 308 000]	250 000 [35 225; 690 500]*	135 000 [14 027; 460 000]
HIV RNA was not detected, n, (M±m, %)	1 (3.2±3.2) %	2 (11.8±7.8) %	4 (8.3±4.0) %
≤100 000, n (M±m, %)	11 (35.5±8.6) %	8 (48.1±12.1) %	18 (37.5±7.0) %
>100 000, n (M±m, %)	19 (61.3±8.7) %	7 (41.2±11.9) %	26 (54.2±7.2) %
$CD4^+$ (median [25 %; 75 %])	220.4 [34.6; 280.5]	197.5 [54.3; 309.0]	185.5 [60.9; 318.0]
≤200, n (M±m, %)	16 (51.6±9.0) %	8 (47.1±12.1) %	25 (52.1±7.2) %
201-350, n (M±m, %)	8 (25.8±7.9) %	5 (29.4±11.0) %	13 (27.1±6.4) %
>350, n (M±m, %)	7 (22.6±7.5) %	4 (23.5±10.3) %	10 (20.8±5.9) %
Correlation $CD4^+/CD8^+$ (mediana [25 %; 75 %])	0.2 [0.1; 0.5]	0.2 [0.1; 0.4]	0.2 [0.1; 0.4]
Duration of HIV infection, years, (mediana [25 %; 75 %])	5.0 [3.5; 8.0]	6.0 [2.5; 7.5]	5.5 [3.0; 8.0]

Notes: * – significant difference between the groups of patients with glomerular and tubulointerstitial diseases (p<0.05-0.001).

respectively, the same as in the total number of HIV-infected people.

According to Table 3, in both groups of patients, fungal diseases were the most common (51.9±9.6) and (52.4±10.9)% respectively, the same as the diseases of viral etiology – (18.5±7.5) and (33.3±10.3)%, respectively, without significant differences in their incidence (p>0.05).

Clinical description of the HIV-infected patients with different levels of proteinuria is presented in Table 4.

According to Table 4, the HIV-infected patients with a level of PU more than 1 g/day were much more frequently diagnosed with arterial hypertension (52.4±10.9) versus (14.8±6.8)% in the patients with proteinuria not exceeding the indicated level (p<0.01). Thus, the relationship between the level of PU and the presence of arterial hypertension was established. It should be noted that according to the level of blood pressure, the patients in the groups were distributed as follows: the first degree AH was diagnosed in 3 (11.1%) patients of the first group and in 3 (14.3%) patients of the second group; the second degree AH was revealed in 2 (7.4%) and 4 (19.0%) patients respectively, and the third degree AH was only found in one (3.7%) patient of the first group and in 3 (14.3%) persons with proteinuria >1 g/day.

The incidence of hypercholesterolemia and hypoalbuminemia in the comparable groups was approximately the same (p>0.05). At the same time, the level of hemoglobin (99.0 [78.0, 123.0]) (median [interquartile scale]) was expectedly much lower in the patients with a higher level of PU vs. (124.0 [93.0; 139.0]) g/l (p<0.05), the incidence of hematuria was much higher as well (90.5±6.4) vs. (51.9±9.6)% (p<0.01).

It is noteworthy that with the increase in PU the GFR levels decreased significantly from (72.0 [38.3; 99.6]) to (48.3 [30.5; 61.8]) ml/min/1.73 m^2 (p<0.01), and only in the group of patients with PU less than 1 g/day the GFR did not drop off below 30 ml/min/1.73 m^2 (Fig. 2).

There were statistically significant intergroup differences in the severity of kidney lesion, depending on the level of proteinuria. For instance, the preserved renal function (GFR≥90 mL/min/1.73 m^2) was more frequently evidenced

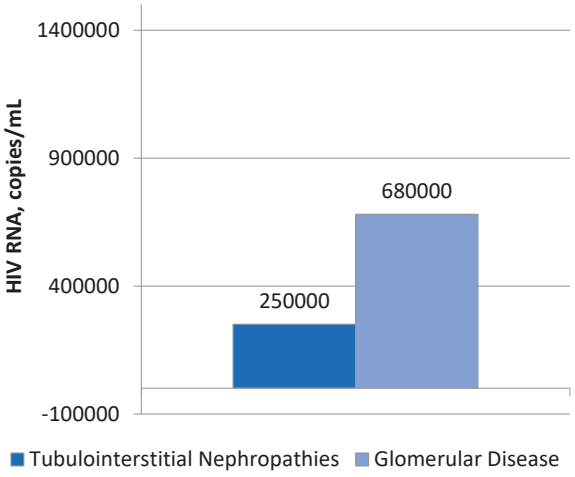

Fig. 1. HIV RNA level in the HIV-infected patients with different variants of kidney damage.

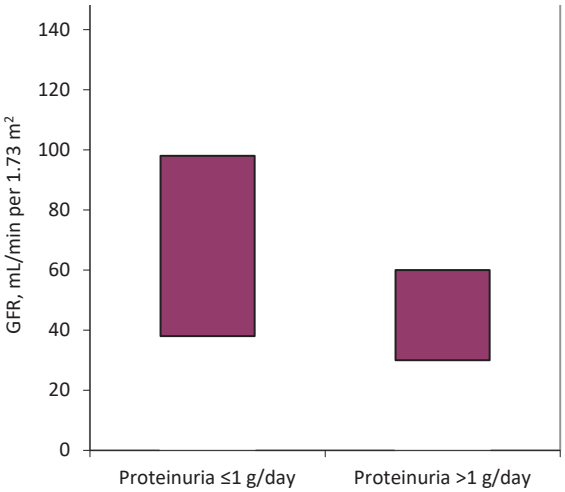

Fig. 2. Correlation among the levels of proteinuria and glomerular filtration rate.

Table 3. Frequency of opportunistic diseases in the HIV-infected patients with different levels of proteinuria

Opportunistic infections	Proteinuria level				p
	≤1 g/day (n=27)		>1 g/day (n=21)		
	n	M±m, %	n	M±m, %	
Bacterial	3	11.1±6.0	2	9.5±6.4	>0.05
Viral	5	18.5±7.5	7	33.3±10.3	>0.05
Fungal	14	51.9±9.6	11	52.4±10.9	>0.05
Parasitic	3	11.1±6.0	2	9.5±6.4	>0.05
Tuberculosis	4	14.8±6.8	6	28.6±9.9	>0.05

Table 4. Clinical and laboratory characteristics of the HIV-infected patients with different levels of proteinuria

Indicator	Proteinuria level		p
	≤1 g/day (n=27)	>1 g/day (n=21)	
Arterial hypertension, n (M±m, %)	4 (14.8±6.8)	11 (52.4±10.9)	<0.01
AT Systolic, mm Hg (median [25%; 75%])	135 [100; 170]	145 [110; 180]	>0.05
Diastolic blood pressure, mm Hg (median [25%; 75%])	90 [85; 100]	95 [90; 110]	>0.05
Cholesterol, mmol/L (median [25%; 75%])	4.1 [3.2; 5.4]	4.3 [3.4; 5.8]	>0.05
Hypercholesterolemia, n (M±m, %)	5 (18.5±7.5)	4 (19.0±8.6)	>0.05
Hypoalbuminemia, n (M±m, %)	7 (25.9±8.4)	7 (33.3±10.3)	>0.05
Albumin, g/l (median [25%; 75%])	36.9 [30.3; 42.8]	34.1 [28.7; 38.5]	>0.05
Hemoglobin, g/l (median [25%; 75%])	124.0 [93.0; 139.0]	99.0 [78.0; 123.0]	<0.05
Hematuria, n (M±m, %)	14 (51.9±9.6)	19 (90.5±6.4)	<0.01
GFR, ml/min /1.73 m^2 (median [25%; 75%])	72.0 [38.3; 99.6]	48.3 [30.5; 61.8]	<0.01
≥90, n (M±m, %)	13 (48.1±9.6)	2 (9.5±6.4)	<0.01
60-89, n (M±m, %)	9 (33.3±9.1)	3 (14.3±7.6)	>0.05
30-59, n (M±m, %)	5 (18.5±7.5)	10 (47.6±10.9)	<0.05
15-29, n (M±m, %)	0 (0.0±0.0)	3 (14.3±7.6)	<0.05
<15, n (M±m, %)	0 (0.0±0.0)	3 (14.3±7.6)	<0.05

in the patients of the 1st group (48.1±9.6)% and much less frequently in those with proteinuria >1 g/day – (9.5±6.4)% (p<0.01). On the contrary, the GFR, which corresponded to the 3rd stage of CKD, was evidenced in half of patients with proteinuria >1g/day (47.6±10.9)% and only in 18.5±7.5% of patients with PU ≤1 g/day (Fig. 3). Accordingly, the terminal renal insufficiency (GFR<15 mL/min/1.73 m^2) was revealed only in the patients of the 2nd group, in 2 of them proteinuria exceeded 3.0 g/day.

The mean reciprocal correlation between the levels of proteinuria and the glomerular filtration rate (r=-0.562, p<0.01), as well as between the levels of proteinuria and hemoglobin (r=-0.596, p<0.01) have been also established.

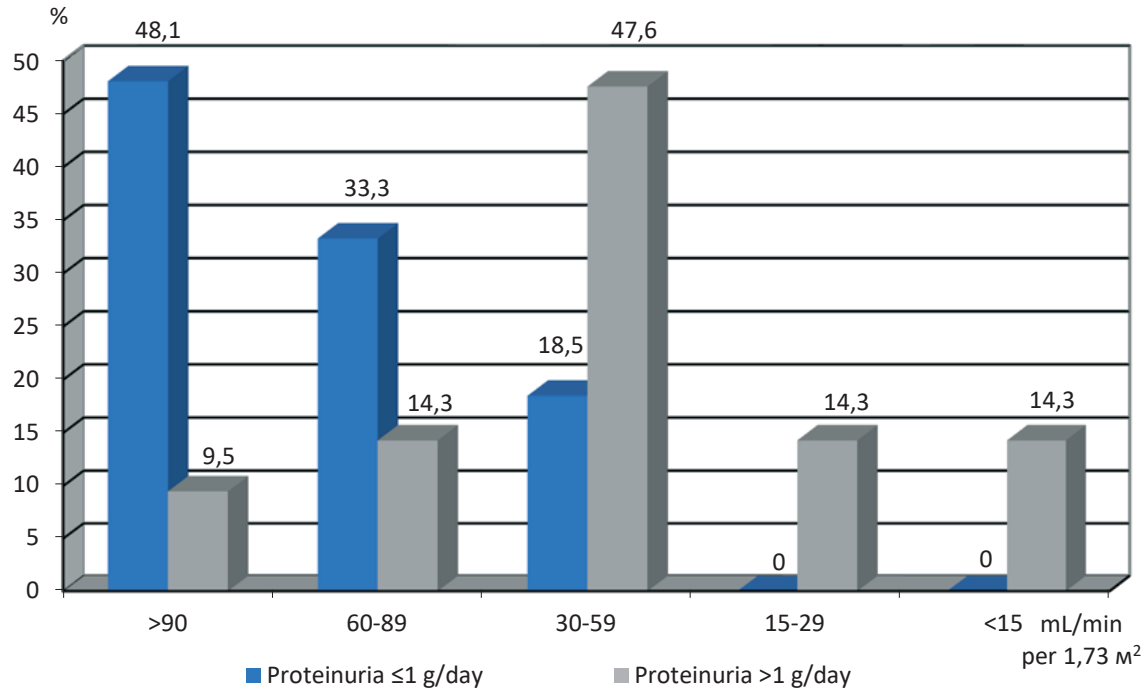

Fig. 3. The morbidity with CKD in the HIV-positive patients depending on the level of proteinuria and glomerular filtration rate.

Discussion

Thus the clinical manifestations of kidney lesion in the studied patients coincide with the typical ones for various pathologies in the general number of nephrology patients.

There are numerous experiments on the study of a number of renal diseases in the HIV-infected individuals worldwide [4, 8, 9]. For instance, the studies conducted in the United States have revealed that, according to the renal biopsy, 52.7% of the patients with nephrotic PU were diagnosed with HIV-associated nephropathy. They were all African Americans. The high incidence of this pathology is associated with racial affiliation, as well as with a specific variant of the antigen/receptor to Duffy chemokines, which are found in the renal tissue [10]. According to the results of multicenter studies in France and Italy, where most patients were of the Caucasian race, among morphologically verified diagnoses, immune deposit diseases were prevalent in the HIV-infected patients with kidney pathology [11, 12].

Proteinuria is established to be one of the major laboratory criteria for CKD. Therefore, the next stage of the work was the establishment of clinical and laboratory features of renal impairment depending on the level of protein in urine.

Thus, the analysis proved that the increase in PU levels was accompanied by a significant renal dysfunction and a more frequent combination with arterial hypertension and hematuria without significant differences in the frequency of opportunistic diseases. The inverse correlation between the level of proteinuria, GFR and hemoglobin value has been established.

According to other indicators characterizing the course of HIV infection in people with different clinical variants of chronic kidney lesion, there were no reliable differences.

Conclusions

Kidney lesions in HIV-infected are most often characterized by tubulointerstitial lesions. At the same time, glomerular kidney lesion, which is much less common, is accompanied by a significantly higher level of HIV RNA.

An increase in proteinuria level is accompanied by a significant renal dysfunction and a more frequent combination with arterial hypertension and hematuria without significant differences in the incidence of opportunistic diseases. The mean reciprocal correlation between the levels of proteinuria and glomerular filtration rate (r=-0.562, p<0.01), as well as between the levels of proteinuria and hemoglobin (r=-0.596, p<0.01) have been established as well.

УРАЖЕННЯ НИРОК У ВІЛ-ІНФІКОВАНИХ

М. О. Андрущак

БУКОВИНСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ, ЧЕРНІВЦІ, УКРАЇНА

Вступ. *Однією з найважливіших проблем сучасності є епідемія ВІЛ-інфекції. За 30-річну історію цієї хвороби ВІЛ уразив понад 75 мільйонів людей, з них майже 30 мільйонів дорослих і дітей померли. У найближчі десятиліття, як і раніше, вони відіграватимуть істотну роль у світових показниках передчасної смертності.*

Мета роботи – *встановити клінічні та лабораторні особливості ураження нирок при ВІЛ-інфекції.*

Методи. *Обстежено 292 хворих на ВІЛ-інфекцією, які перебували на амбулаторному спостереженні в Чернівецькому обласному центрі з профілактики та боротьби зі СНІДом. На підставі основних маркерів пошкодження нирок (персистентна протеїнурія та швидкість клубочкової фільтрації <60 мл/хв/1,73 м^2) у 48 осіб діагностовано хронічну хворобу нирок, яка з великою частотою супроводжувалася порушенням функції цих органів.*

Результати *Встановили, що у ВІЛ-інфікованих ураження нирок найчастіше характеризується їх тубулоінтерстиційним ураженням. Водночас гломерулярне ураження нирок, що буває значно рідше, супроводжується достовірно вищим рівнем РНК ВІЛ.*

Підвищення рівня протеїнурії супроводжувалося достовірно значущим порушенням функції нирок і частішим поєднанням з артеріальною гіпертензією і гематурією за відсутності достовірних відмінностей у частоті опортуністичних захворювань. Встановлено зворотну середньої сили кореляцію між рівнями протеїнурії і швидкістю клубочкової фільтрації – (r=-0,562, p<0,01), а також між рівнями протеїнурії та гемоглобіну (r=-0,596, p<0,01).

Висновки *У ВІЛ-інфікованих ураження нирок найчастіше характеризується їх тубулоінтерстиційним ураженням. Водночас гломерулярне ураження нирок, що буває значно рідше, супроводжується достовірно вищим рівнем РНК ВІЛ.*

КЛЮЧОВІ СЛОВА: **ВІЛ-інфекція; хронічна хвороба нирок; тубулоінтерстиційне ураження; гломерулярне ураження нирок.**

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PREVALENCE OF GESTATIONAL DIABETES MELLITUS IN AN URBAN INDIAN COHORT USING DIABETES IN PREGNANCY STUDY GROUP IN INDIA (DIPSI) CRITERIA – VALIDATING ONE-STEP APPROACH

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Background. *India is the "World's Diabetes capital", with half the diabetic population being women. Early detection of glucose intolerance during pregnancy offers a timely opportunity for screening, management and prevention of gestational diabetes mellitus (GDM) and prevents fetal complications.*

Objective. The study assessed the prevalence of GDM in an Indian cohort using the Diabetes in Pregnancy Study group of India (DIPSI) criteria.

Methods. *200 pregnant women underwent two-phase testing with non-fasting 75-gram glucose challenge under Diabetes in Pregnancy Study group of India (DIPSI) criteria at <20 weeks and between 24-28 weeks period of gestation. A 3-hour 100-gm oral glucose tolerance test (OGTT) was used for confirmation. Repeat testing was done for women negative during the first-phase.*

Results. *Mean age was 24.26±3.75 years with 52.5% multigravidas. Mean Body Mass Index (BMI) was 20.7±3.07 kg/m^2. The prevalence of GDM in study cohort was found to be 15.5% using the DIPSI criteria while the prevalence of GDM after 100 g OGTT was 13.0%. GDM was mostly seen to occur in women of 26-30-year age group. Statistically significant associations for age and GDM, and BMI and GDM were evidenced.*

Conclusions. *Maternal age of ≥25 years should be adopted as a risk factor for the development of GDM. The DIPSI criteria offer a cost-effective and an evidence-based protocol for a single-step definitive glucose test for both screening and diagnosis of pregnant patients belonging to any socio-economic strata; furthering its implementation for public health obstetrics.*

KEY WORDS: **gestational diabetes mellitus; DIPSI criteria; screening; pregnancy; glucose tolerance test.**

Introduction

Gestational Diabetes Mellitus (GDM) is established to be carbohydrate intolerance with onset or first diagnosis during pregnancy [1]. The WHO defines GDM as plasma glucose concentration of >140 mg/dl 2-hours by 75-gm oral glucose tolerance test (OGTT) similar to that of impaired glucose tolerance (IGT) test in a non-pregnant state [2]. With advancement of pregnancy, insulin resistance and diabetogenic stress caused by placental hormones necessitates compensatory increase in insulin secretion, the inadequacy of which leads to the development of GDM. The patients with GDM are at a risk group of future diabetes mellitus (DM) development, predominantly type-2 DM, as well as their children are [3]. In addition, untreated GDM may possibly lead to increased risk of large for gestational age fetus, plunge in blood sugar and jaundice in the offspring.

The prevalence of DM is increasing worldwide. Developing countries sustain a major proportion of world population translating to the epidemic proportions of DM being encountered by the healthcare fraternity in limited resource public health infrastructure. India is projected as the "World's Diabetes capital", with half the diabetic population being women. India is expected to contain the highest population of diabetics by 2025. The syndemic (synergistic epidemic) of DM and obesity compounding the problem of GDM exists under socio-epidemiological and anthropological perspectives of health disparity factored by poverty, living conditions, socio-economic status and dietary habits.

GDM is the most common metabolic disease of pregnancy worldwide. The prevalence of GDM reaches up to 14% of all pregnancies, resulting in approximately 200,000 cases annually in the United States. Asian and Indian

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lifestyles are starkly different from Western lifestyles translating into 11.3 times higher relative-risk of GDM in Indian women compared to their western counterparts [4].

With the population experiencing a changing lifestyle and epidemiology of DM, it is pertinent to offer screening of GDM during the antenatal work-up. GDM holds out a significant opportunity for testing, development and implementation of clinical strategies for diabetes prevention in people [5]. Timely screening of the pregnant women for glucose intolerance, succeeding euglycemia and adequate nutrition may prevent presumably the pathological cycle of vertical transmition of glucose intolerance. This necessitates the universal mandatory screening for GDM during pregnancy, which is a resource intensive concept in the developing country perspective. Presently most institutions catering to women with adequate affordability are following the 2-phase procedure for screening GDM. The criteria by Diabetes in Pregnancy Study Group of India (DIPSI) recommend a simplified one-step approach for the screening and diagnosis of GDM irrespective of fasting state of expectant mothers, which is a promising protocol for underprivileged communities having limited healthcare accessibility during pregnancy.

Timely revealing of glucose intolerance in pregnancy offers an opportunity for screening, management and prevention of GDM on time and prevents fetal complications thus improves neonatal outcomes [6, 7]. This necessitates the general mandatory screening for GDM during pregnancy, which is a resource intensive testing modality. This study was carried out to assess the incidence of GDM in an Indian cohort using the DIPSI criteria [8].

Methods

The triple-blind study was conducted amongst 200 patients admitted to the antenatal outpatient department (OPD) of a tertiary-care hospital, containing 1600 beds, and medical teaching institute in Western India; the study was approved by the Ethics Committee of these medical facilities as well as the written informed consents were attained from the patients. All pregnant females at 20 weeks or less period of gestation (POG) were involved in the study that lasted for two years: from May 2012 to Apr 2014. The patients with GDM/Impaired Glucose Tolerance (IGT) in previous pregnancy, established morbidity of DM, DM in a first-degree relative or with a history of unexplained stillbirth, large for gestational age offspring, congenital anomalies or previous birth injuries, were excluded. Relevant history, general examination for calculating body-mass index and evidence of insulin resistance along with obstetric/gynecological examination were carried out. Triple-blinding of a patient, gynecologist and laboratory medicine specialist was ensured to eliminate bias and confounding.

The entire cohort of 200 patients was subjected to a two-phase testing at the POG of <20 weeks and for a second time at the POG of 24-28 weeks, a temporal separation was at least four weeks. In the first phase, all patients were given 75-gm anhydrous oral glucose at their first visit, irrespective of their fasting state, according to the DIPSI criteria. The levels of plasma venous blood glucose were evaluated by glucose oxidase-peroxidase method in 2 hours. The indices of ≥140 mg/dl were positive by the DIPSI criteria. A 3-hour 100-gm oral glucose tolerance test (OGTT) was used for confirmation. Any indices of ≥95 mg/dl fasting, ≥180 mg/dl in 1 hour, ≥155 mg/dl in 2 hours, ≥140 mg/dl in 3 hours were considered to be positive. Only one positive value in OGTT was considered as IGT while two positive values were considered for GDM.

In the second phase, women who were negative initially by DIPSI criteria were made to undergo a repeat test with non-fasting 75-gm at 24-28 weeks as per the DIPSI criteria. A 100-gm OGTT was used for confirmation.

Data was analyzed using SPSS (version 21; IBM Corporation) with χ^2 test or Fisher's exact test for categorical variables and Student's t-test for continuous variables. All statistical tests were two-tailed and P values <0.05 were considered significant. Clinicodemographic and diagnostic profiles were correlated for descriptive statistics and included frequency, percentages and 95% confidence intervals (95% CI).

Results

The study cohort comprised of young patients with mean age was 24.26±3.75 years ranging from 20 to 28 years. Most patients were between 21 to 25 years of age (102/200, 51%, 95% CI 43.87% – 58.09%), followed by 49/200, 24.5%, 95% CI 18.83% – 31.17%, between 26 to 30 years (24.5%) (Table 1).

95/200, 47.5%, 95% CI 40.45% – 54.65% were primigravida while 105/200, 52.5%, 95% CI 45.35% – 59.55% were multigravida. Mean Body Mass Index (BMI) was 20.7 ± 3.07 kg/m^2, range

between 14.33 to 30.81 kg/m^2. Most of the pregnant females (108/200, 54%, 95% CI 46.83% – 61.01%) were having BMI between 21-25 followed by 92/200 (46%, 95% CI 38.99% – 53.17%), who had BMI ≤20 kg/m^2. There were no overweight or obese women in the cohort (Table 2). The study was carried out with a 100% follow up with no drop outs.

Out of the 200 pregnant females in the cohort, in the first phase, 31/200 (15.5%, 95% CI 10.93% – 21.44%) were tested positive by the DIPSI criteria prior to 20 weeks POG; 21/200 (10.5%, 95% CI 6.77% – 15.81%) of them were tested positive by 100-gm OGTT. In the second phase, the remaining 10 women tested positive by the DIPSI criteria and negative by 100-gm OGTT were again subjected to 100-gm OGTT at a 24-28-week POG, resulting in five more being found positive by 100-gm OGTT.

Out of the 169 women tested negative by the DIPSI criteria at less than 20 weeks POG, in the first phase, one aborted at 14 weeks POG and was excluded from the study. The remaining 168 women were again subjected to DIPSI and then validated by 100-gm OGTT at a 24-28-week POG. None tested positive with either DIPSI or 100-gm OGTT.

The prevalence of GDM in study cohort was found to be 15.5% using DIPSI criteria while the prevalence of GDM after 100-gm OGTT was 13% (Table 1). GDM was mostly seen to occur in women of 26-30-year age group (12/26, 46.15%, 95% CI 27.14% – 66.25%) followed by 9/26 (34.62%, 95% CI 17.95% – 55.64%) in the 21-25-year age group. Statistically significant association for age and GDM (p=0.003) was seen by Fisher's exact test. Almost all (25/26, 96.15%, 95% CI 78.41% – 99.8%) GDM was seen with BMI >20 kg/m^2, with statistically significant (p=0.003) difference seen by Fisher's exact test. However, the association of gravidity was not significant (p=0.207) using Chi square test.

Discussion

Disorders of maternal glucose metabolism during pregnancy are two-pronged. Firstly, pre-existing type-2 DM accounts for 8% of DM in pregnancy. There is an increasing trend of type-2 DM in women of childbearing age group, attributable to sedentary lifestyles, dietary changes and the virtual epidemic of adolescent and childhood obesity.

GDM accounts for 90% of diabetes in pregnancy. GDM represents the "tip of an iceberg" for the overall prevalence of DM in the population, thus being representative screening target for timely intervention. The prevalence of GDM varies from 1-20% depending upon population sample and diagnostic criteria.

Risk factors of GDM include a high BMI (a measure of body fat), gaining weight or low physical activity in pregnancy, excessive dietary eating of polyunsaturated fats, glucose intolerance (a sign of diabetes) or delivery of a large baby in previous pregnancies, as well as a family history of diabetes. Excessive intake of saturated fat, low eating of polyunsaturated fat, and high gestational weight gaining may possibly increase the risk of GDM. A decreased risk of GDM is also associated with physical activity. Obesity is one of the most significant risk factors for GDM, its prevalence has been increasing much over the last decades [9, 10, 11].

The study revealed GDM among young pregnant females up to 30 years of age in contrast to the development of DM in later age. The risk of GDM increases significantly from 25 years onwards [12]. The most predictive factor of GDM is maternal age ≥25 years, according to the recommendations of the American Diabetes Association (ADA) on the age criteria of ≥25 years as a cut-off for screening for GDM. In population with lower diabetes prevalence, timing of screening depends on the risk profile. Women at high risk are offered screening at

Table 1. Age profile of pregnant patients (n=200)

Age (years)	Number of patients	Percentage (%)	95% confidence intervals
≤20	35	17.5	12.64-23.64%
21-25	102	51.0	43.87-58.09%
26-30	49	24.5	18.83-31.17%
>30	14	7.0	4.03-11.71%

Table 2. Body-mass index (BMI) profile of pregnant patients (n=200)

BMI (kg/m^2)	Number of patients	Percentage (%)	95% confidence intervals
≤20	92	46	38.99-53.17%
21-25	108	54	46.83-61.01%
>26	0	0	–

first antenatal visit, moderate risk at 24-28 weeks as per ADA guidelines. In general, screening and diagnostic tests are performed between 24 and 28 weeks, because at this point in gestation the diabetogenic effect of pregnancy is manifested. The study of Kaiser Permanente of Colorado (KPCO) proved a strong cohort influence on the prevalence of GDM. Regardless of the age and ethnicity, the women, who were born more recently, were at an increased risk for GDM diagnosis compare to those born earlier. This finding most likely reveals an increased exposure to risk factors taking place before childbearing age [13]. In clinical practice, maternal age of ≥25 years should be adopted instead of ≥35 years or ≥40 years as a risk factor for the development of GDM [14, 15].

The present study also proved that the increased prevalence of GDM was evidenced together with increasing BMI. Although the incidence of GDM in the pregnant females with normal weight (BMI 18.5-24.9) is 2.3%, it increased more than five-fold to reach 11.5% in extremely obese pregnant women with BMI 35-64.9 [16, 17, 18]. A systematic review of observational studies published over last 30 years, which elected maternal BMI as the only measure of obesity and where all diagnostic criteria for GDM were accepted; it revealed that for every 1 kg/m^2 increase in BMI, the prevalence of GDM increased by 0.92% (95% CI 0.73% to 1.10%) [19]. Indian women with GDM experience a higher risk of metabolic syndrome and diabetes [20].

The 15.5% prevalence of GDM by DIPSI criteria found in this study compares well to other Indian studies showing prevalence between 16.55% and 22%. In India the prevalence of GDM has been estimated at 16.55% by the WHO criteria of a 2-hour blood glucose level of 140 mg/dl. However, the prevalence for Kashmiri women was 3.8% [21, 22, 23, 24, 25]. GDM was proved to be more widespread in urban areas than in rural. For this population and ethnicity, the incidence of GDM corresponds to the incidence of IGT in non-pregnant adults within that population [26]. In Indian context the prevalence of GDM is steadily increasing from 2% in 1982 to 12% in 1991 to 16.55% in 2002. Variations in prevalence of GDM due to geography and ethnicity have similarly been reported in Mexico [27].

Certain ethnically diverse subpopulations have a much higher rate of GDM which renders them the susceptibility of a greater predisposition to DM in later age. The incidence of GDM differs in direct share to the incidence of type-2 DM for tis ethnic group or population. In Asian population, GDM reflects the prevalence of IGT in the population. Therefore, the general screening for GDM is necessary for Asian and Indian population [28]. In comparison to the selective screening, the general one for GDM distinguishes more patients and improves neonatal and maternal prognosis. Currently, and after extensive deliberation, universal screening of all pregnant women is recommended by some professional associations. Nevertheless, there exist challenges in quality control of laboratory testing in developing countries catering to mass-screening in resource limited laboratories, which needs to be taken into account for clinical decision making [29, 30].

In pregnancy, the choice to carry out a placebo-controlled trial involves clinical equipoise [31]. Hence, there was no control group of unmanaged pregnant women in this study, as there are some publications confirming that management of GDM women, as defined by the WHO criteria, was associated with a decreased risk of pregnancy outcome. As the routine screening for glucose intolerance during pregnancy was not done initially, probably the undiagnosed glucose intolerance that was occurring in the past has resulted in the increased prevalence of diabetes in India.

DIPSI criteria are a major advance as they cater diagnosis and screening of all pregnant women regardless of the fasting state by a single-step approach with a 75-gm of a 2-hour glucose test and a cut-off of >140mg/dl for diagnostics. The study revealed 31 patients through DIPSI criteria at ≤20 weeks POG, 21 of which were detected by 100-gm OGTT at ≤20 weeks POG and five were detected between 24-28 weeks POG. If the 75-gm criterion was reapplied at 32-34 weeks POG as recommended by DIPSI, it is likely that even the five women, who tested negative, when validated with 100-gm OGTT, could have tested positive for GDM. DIPSI is very economical, practical, convenient and feasible for patients and obstetric healthcare practitioners [32, 33].

Conclusions

The incidence of Gestational diabetes mellitus in the study cohort using DIPSI criteria was significantly high (15.5%) and is comparable with other Indian studies. In clinical practice, the maternal age of ≥25 years instead of 35

years or ≥40 years should be adopted as a risk factor for GDM development. The DIPSI criteria offer a cost-effective and an evidence-based protocol for a single-stage complete glucose test for both screening and diagnosis of pregnant patients of any socio-economic strata; furthering its implementation for public health obstetrics.

ПОШИРЕНІСТЬ ГЕСТАЦІЙНОГО ЦУКРОВОГО ДІАБЕТУ СЕРЕД КОГОРТИ МІСЬКОГО ІНДІЙСКОГО НАСЕЛЕННЯ ЗА КРИТЕРІЯМИ DIPSI – АПРОБУВАННЯ ОДНОЕТАПНОГО ПІДХОДУ

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Вступ. *Індія є «столицею діабету у світі», при чому половину населення, хворого на діабет, складають жінки. Раннє виявлення порушення толерантності до глюкози під час вагітності дає можливість своєчасно проводити скринінг, лікування та профілактику гестаційного цукрового діабету (ГЦД) та запобігати розвиткові ускладнень вагітності.*

Мета дослідження *– встановити частоту розвитку ГЦД серед жінок з використанням критеріїв DIPSI.*

Методи дослідження. *Обстежено 200 вагітних жінок, яким проводили двофазне тестування навантаженням глюкозою (75 г глюкози натще серце) відповідно до критеріїв DIPSI на термінах <20 тижнів та між 24-28 тижнями вагітності. Тригодинний пероральний глюкозотолерантний тест (ПГТТ) (з навантаження 100г глюкози) використовували для повторного дослідження, яке проводили в тому числі і жінкам з негативними результатами, отриманими під час першої фази обстеження.*

Результати. *Середній вік обстежуваних вагітних жінок склав (24,26±3,75) років, з них з 52,5 % – мали кілька вагітностей. Середній індекс маси тіла (ІМТ) становив (20,7±3,07) кг/м². Встановлено, що поширеність ГЦД у досліджуваній когорті становила 15,5 % відповідно до критеріїв DIPSI, тоді як поширеність ГЦД після 100 г ПГТТ становила 13 %. ГЦД в основному спостерігався у жінок вікової групи 26-30 років. Встановлено статистично достовірні кореляції між показниками віку та ГЦД, індексу маси тіла та ГЦД.*

Висновки. *Вік майбутньої матері більше 25 років повинен розглядатися як фактор ризику розвитку ГЦД. Критерії DIPSI – це економічно ефективний і обґрунтований протокол для викориmтання глюкозотолерантного тесту для скринінгу та діагностики вагітних пацієнток, що належать до будь-яких соціально-економічних верств, який може бути рекомендований для подальшої імплементації у клінічну практику.*

КЛЮЧОВІ СЛОВА: **гестаційний цукровий діабет; критерії DIPSI; скринінг; вагітність; глюкозотолерантний тест.**

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BULLOUS PEMPHIGOID A RARE AUTOIMMUNE DISEASE: A CASE REPORT

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Background. *Bullous pemphigoid (BP) is a rare autoimmune blistering skin disease in the elderly and it is manifested by cutaneous blisters on the skin lesions.*

The objective was to emphasize the rare case of BP.

Methods. *A case report of BP in a 58-year-old male patient admitted to a dermatology ward is presented.*

Results. *A 58-year-old male patient with complaints of fluid-filled skin lesions, was examined initially over the trunk, gradually progressed involving B/L upper and lower extremities. Even though the patient was treated with the recommended therapy of corticosteroid (Dexamethasone) along with adjuvant drugs, new skin lesions continued to develop, and the patient's condition worsened. The Prednisolone was started in place of Dexamethasone on the fifth day of treatment at its higher dose (50mg/day), the Prednisolone proved its efficacy to combat the extensive condition of BP.*

Conclusions. *Bullous pemphigoid is a distressing blistering skin disease. Untreated disease is often fatal because of the susceptibility to infection and fluid-electrolyte disturbances. The mortality of patients with bullous pemphigoid has been significantly reduced with the advent of new therapies and treatment modalities. The treatment with systemic and topical corticosteroids forms the mainstay of treatment along with other adjuvant drugs. In the present case study, the use of Prednisolone has proven its efficacy in the extensive disease state of BP and improved the patient's quality of life.*

KEY WORDS: **Bullous Pemphigoid; rare autoimmune disease; Dexamethasone; Prednisolone.**

Introduction

Bullous pemphigoid (BP) is a rare autoimmune blistering disease and affects the elderly mostly. However, on rare occasions, it may affect children and young adults too. The annual incidence of BP has been estimated to range from 2 to 14 new cases per one million people. Its incidence is expected to rise as a consequence of population aging. A recent study in France has established a 3-time increase in the annual incidence of BP over the last 15 years with 21.7 new cases per million inhabitants [1]. Although several clinical variants have been recognized, BP usually presents with tense blisters arising on healthy or erythematous skin, typically involving the flexor surfaces. Autoantibodies against well-characterized autoantigens, BP180 and BP230, are believed to play a crucial role in the pathogenesis of BP. The prognosis of BP has been studied by several research groups, but the results of the studies have been inconsistent [2]. The body's immune system is confused and makes an antibody (a type of protein used to fight infection) that targets a part of the skin that normally holds it together. The attack on the skin causes blisters (firm, fluid-filled bubbles on the skin) to form. This disease most often involves only the skin, but sometimes it may affect to eyes, mouth, and genital organs. The following clinical variants are described: classic (described above), localized, nodular, vegetating, erosive, erythrodermic, juvenile and drug-induced [3].

The clinical presentation of BP is tense blisters, which are often seen on erythematous or normal-looking skin of limbs and trunk and may be widespread or localized. Bullae and/or erosions may be present in the oral and genital mucosa. Pruritus alone or associated with erythema and/or urticated plaques may precede the formation of bullae by weeks or months; in some cases, bullae may not become clinically apparent [4]. A recent study conducted in the

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United Kingdom found an incidence of 4.3 (95% confidence interval (95% CI), 4.0–4.6) per 100,000 person-years [5]. The BP patients have complex co-morbidity profiles, most notably neurological disorder, as well as autoimmune and are prone to infection. Various studies have confirmed a strong association between BP and neurological disorders [6].

Case study

A 58-year-old male patient admitted to a dermatology ward with chief complaints of fluid-filled skin lesions (Fig. 1), initially over the trunk, gradually progressed involving bilateral upper and lower extremities. The lesions did not rupture spontaneously and were associated with mild itching. Upon admission, general physical examination was performed in which the patient was moderately built and nourished, conscious and co-operative. Blood pressure was 80/60 mm Hg. Local examination of the skin was performed. It showed that multiple tense vesicles and bullae over the trunk, on both sides upper and lower extremities, few erosions, Nikolsky's sign, and bulla signs had spread. The initial blood investigation revealed total count (TC) 19970 cells/mm^3, neutrophils 57.4%, lymphocytes 15.5%, eosinophils 24.5%, monocytes 2.3%, basophils 0.3%, RBC 5.05 $\times 10^{12}$/l, Hb 15.0 g/dl, Packed Cell Volume 45.3%, MCV 89.7 fl, MCH 29.7 pg, MCHC 33.1 %, platelet count 240000 cells/mm^3, ESR 10 mm/hour.

Initially, dexamethasone IV and oral were prescribed along with other drugs: Ranitidine, Calcimax (Calcium+ Vitamin D_3+ Magnesium + Zinc) and Teczine (Levocetirizine). In two days, a few new lesions occurred, then tablet CefiXL (Cefixime+Cloxacillin) 200 mg was prescribed. In 24 hours, multiple new lesions occurred again, then Omnacortil (Prednisolone) (higher dose) – a corticosteroid, was prescribed. GV lotion (Gentian Violet) and Ointment liquid paraffin were prescribed twice a day (BID), as well as tablet Dapsone (Dapsone) once daily (OD) and Capsule Nicoglow (Nicotinic acid) BID. Treatment was continued up to the time of discharge and the patient condition improved.

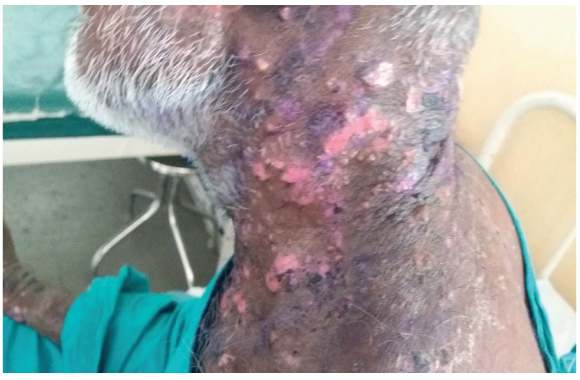

Fig. 1. Skin lesions of Bullous pemphigoid.

Discussion

Bullous pemphigoid is a rare autoimmune blistering disease; it typically affects the elderly and is followed by significant morbidity and mortality [7]. The clinical symptoms of the disease are development of oral lesions in about one-third of the patients; lesions may occur on the trunk, extremities, and intertriginous areas. In most of the cases, no clear precipitating factors are identified; some precipitating factors are exposure to ultraviolet light, radiation therapy and exposed to certain drugs like furosemide, penicillin, sulfasalazine, and captopril [7]. The disease is characterized by the formation of IgG auto antibodies targeting dystonin (bullous pemphigoid antigen 1 (BPAG1), and /or type XVII collagen also called bullous pemphigoid antigen 2 (BPAG2), which is a component of hemidesmosomes [2]. In the present case fluid-filled lesions were seen initially over the trunk and gradually progressed involving lower and upper extremities.

The recommended treatment for BP is as follows [8]:

Initial therapy. Initial therapy is determined by the extent and rate of progression of the lesions. The priority is to control lesions usually in a slowly progressive form of the disease; initial treatment includes intralesional injections of corticosteroids or topical applications of corticosteroids.

Maintenance therapy. Once most lesions are healed, the dose and type of medication are gradually reduced to limit the risk of side effects. Understanding the rate of dose reduction is determined by clinical response and overall disease activity. It is important to monitor this balance and limit use of unnecessary medication as many fatalities are related to complications associated with the therapy.

The available treatments work via different mechanisms. Some aim to suppress the inflammatory process e.g. corticosteroids, antibiotics, anti-inflammatory mediators. Immune modulating treatments include intravenous immunoglobulins. Intravenous immunoglobulin has been widely tried as an immunomodulatory agent in various auto-antibody mediated blistering diseases.

Systemic corticosteroids are most commonly used: Dexamethasone and Prednisolone. Typical recommendations for wide spread disease are for a starting daily dose of about 1 mg/kg continued until cessation of new blister formation then gradually decreased. The starting dose ranges between 40 and 80 mg daily, usually 60 mg daily. Lower starting doses of 20 to 40 mg daily have been recommended. Antibiotics should be considered as the first line of treatment for both localized and mild to moderate disease. Antibiotic treatment is provided for at least 2 weeks. Azathioprine and methotrexate are also recommended [8].

In the present case study, the patient was prescribed with Dexamethasone initially for four days, even though a few new lesions developed. In the next consecutive days the Dexamethasone was replaced with Prednisolone at its higher dose, and then the patients' condition improved. Similar kind of results was noticed with Prednisolone prescription in the extensive disease state of BP [7]. Dapsone was given in one week after patient's admission as it was an immunosuppressant to reduce the action of auto-antibodies and nicotinamide was given on the last two days as it had some effects in reducing itching and redness.

Conclusions

Bullous pemphigoid is a distressing blistering skin disease. Untreated, Bullous pemphigoid is often fatal because of the susceptibility to infection and fluid-electrolyte disturbances, hence utmost importance is given for BP treatment. The mortality of patients with bullous pemphigoid has been significantly reduced with the advent of new therapies and treatment modalities. The treatment with systemic and topical corticosteroids forms the mainstay of treatment of BP along with other adjuvant drugs. In the present case study, the use of Prednisolone has proven its efficacy in the extensive disease state of BP and improved the patient's quality of life.

БУЛЬОЗНИЙ ПЕМФІГОЇД – РІДКІСНЕ АВТОІМУННЕ ЗАХВОРЮВАННЯ (КЛІНІЧНИЙ ВИПАДОК)

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Вступ. *Бульозний пемфігоїд (БП) – це рідкісне автоімунне захворювання шкіри у літніх людей, яка проявляється епідермальними пухирями на пошкоджених шкірних покривах.*

Мета – *акцентувати увагу на рідкісному випадку БП.*

Методи дослідження. *Представлено клінічний випадок БП у 58-річного пацієнта-чоловіка, який поступив у дерматологічне відділення.*

Результати дослідження. *Обстежено 58-річного пацієнта із скаргами на заповнені рідиною утвори на шкіри, які з'явилися спочатку на тулубі, і поступово прогресували, поширюючись білатерально на верхні та нижні кінцівки. Незважаючи на те, що пацієнт отримував рекомендовану терапію на базі кортикостероїду (дексаметазон) разом з ад'ювантними препаратами, шкірні елементи та пошкодження шкіри прогресували, і стан хворого погіршився. Було застосовано преднізолон замість дексаметазону на п'ятий день лікування у більш високій дозі (50 мг/добу). Останній довів свою ефективність для боротьби з прогресуючим БП.*

Висновки. *Бульозний пемфігоїд – хронічне, набуте аутоімунне захворювання. Нелікована хвороба часто смертельна через схильність до інфекцій та порушення водно-електролітного обміну. Смертність пацієнтів з бульозним пемфігоїдом значно зменшилася з появою нових методів терапії. Базисним є лікування системними і топічними кортикостероїдами У цьому дослідженні застосування преднізолону довело свою ефективність при генералізованій формі захворювання та дозволило поліпшити якість життя пацієнта.*

КЛЮЧОВІ СЛОВА: **бульозний пемфігоїд; рідкісні аутоімунні захворювання; дексаметазон; преднізолон.**

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PREVALENCE AND RISK FACTORS FOR VITAMIN D DEFICIENCY IN OVERWEIGHT AND OBESE ADOLESCENTS IN UKRAINE

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Background. *Vitamin D affects the function of many organs and systems. Lipid metabolism disorder is established to be one of the risk factors for vitamin D deficiency, and the amount of adipose tissue is crucial.*

Objective. *The aim of the study was to determine the prevalence and risk factors for vitamin D deficiency in overweight and obese adolescents.*

Methods. *146 children with excessive weight and obesity as well as 63 healthy children with normal body weight were examined. In the study groups, there were no children taking vitamin D. Vitamin D status was evaluated by the level of 25(OH)D in blood serum. Vitamin D deficiency was diagnosed at the level of 25(OH)D between 20 and 29 ng/ml, and significant deficiency – below 20 ng/ml, normal calcidiol content was 30-100 ng/ml.*

Results. *The average level of 25(OH)D in the adolescents with normal body weight was 19.76±4.28 ng/ml, in the adolescents with excessive body weight – 15.24±3.47 ng/ml, and in the obese children – 13.87±2.71 ng/ml. The prevalence of vitamin D deficiency in the overweight adolescents was 70.62%, and in the adolescents with obesity – 77.19%.*

Conclusions. *Vitamin D deficiency is prevalent in the adolescents with overweight and obesity. To prevent the development of hypovitaminosis and vitamin D deficiency, it is necessary to carry out educational activities with adolescents for promotion of healthy lifestyle and healthy food, as well as to develop an optimal program for improving vitamin D status in the obese children.*

KEY WORDS: **vitamin D; children; calcidiol; prevalence; obesity**.

Introduction

Vitamin D, due to the biological properties of its derivatives, affects the function of many organs and systems. Vitamin D deficiency leads to a decrease in calcium concentration in blood, impairment of calcium and phosphorus absorption in intestines and kidneys because of its active metabolite 1.25-dihydroxyvitamin D [1]. It has been proved that vitamin-D endocrine system affects electrolytes concentration, cell proliferation, angiogenesis, stimulation of insulin synthesis, inhibition of renin secretion [2, 3, 4].

The presence of interconnections between calcidiol level as well as lipid and carbohydrate metabolism in children [5, 6] has been established. Moreover, special attention is paid by the researchers to the development of cardiometabolic risk factors and their relations with the concentration of calcium in blood and levels of parathyroid hormone in cases of vitamin D deficiency [7, 8].

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The period of puberty is characterized by a rapid, peak increase in bone and muscle mass, and requires higher calcium and phosphorus intake, and, therefore, maintenance of proper levels of vitamin D metabolites in blood plasma [9]. However, adolescents frequently suffer from hypovitaminosis D and are characterized by increased tendency towards a sedentary lifestyle, spending much time at a computer or in front of the TV [10]. Leading a sedentary lifestyle in such children reduces the time spent in sunlight and outdoors, which is a direct risk factor for obesity and vitamin D deficiency [1].

Investigating metabolic abnormalities in children of different ages, researchers identified inverse relationship between vitamin D levels and metabolic factors, in particular, insulin resistance, body mass index, triglyceride levels and total testosterone, and direct relationship with insulin sensitivity [11].

It is established that lipid metabolism disorder is one of the risk factors for vitamin D deficiency, and the amount of adipose tissue is crucial in its metabolism and biological significance [12]. Numerous clinical studies have proved that for those suffering from obesity,

vitamin D intake should be 2-3 times higher than for those with normal body weight. There is a pathogenetic connection between obesity and vitamin D deficiency, since vitamin D is a fat-soluble substance, distributed in the adipose tissue, which leads to decrease in its concentration in plasma [13].

Moreover, attention is drawn to the fact that with the increase in the amount of adipose tissue there is a limitation of the bioavailability of vitamin D, which is associated with its engulfment by adipocytes and deposition in the adipose tissue. Thus, Spanish researchers have established existing relations between low-level serum concentration of 25(OH)D with high triglyceride levels regardless of age, sex, body mass index and physical activity [14].

Childhood obesity is an important public health problem. In Ukraine, 12% of children aged 7 to 17 years old suffer from excessive weight [15], among which about 10% are diagnosed with obesity by body mass index. Moreover, the number of obese children has a positive annual increasing rate.

Taking into consideration the increase in the number of overweight and obese adolescents in Ukraine, it has become necessary to determine the prevalence of vitamin D deficiency among the overweight and obese adolescents and to identify the main factors affecting the vitamin D status of such children.

The aim of the research is to determine the prevalence and risk factors for vitamin D deficiency in the adolescents with excessive weight and obesity.

Methods

The research was conducted in the period of 2016-2018 at the Communal Institution of the Ternopil Regional Council "Ternopil Regional Children Clinical Hospital". The Patient Safety Rules and the Ethical Standards and Procedures for Research Involving Human Beings (2000) have been followed in carrying out the study. In all cases, informed consent has been obtained from the patients and/or their parents.

The research involved on 146 adolescents (78 boys and 68 girls) aged 12 to 17 years old, which, depending on the body mass index (BMI), were divided into two groups: the overweight children and the obese children. The adolescent age of each child was determined according to the Tanner scale (2-5 stages) [5, 12]. The control group consisted of 63 healthy children aged 12-17 years old, who lived in the city of Ternopil and sought medical consultations for various reasons and chronic diseases. None of the causes of seeking medical help and disease affected their growth, body structure, nature of nutrition, physical activity. The experimental groups did not include children, whose obesity was due to endocrine diseases (hypothyroidism, hypercorticism, hypopituitarism, traumas of hypothalamic-pituitary area), taking antiepileptic drugs or glucocorticoids.

All children were Ukrainian (Caucasians) and lived in Ternopil region, Ukraine. In anthropometric studies, body height and weight were determined, and BMI was calculated according to the formula (mass (kg)/height^2 (m^2)).

Anthropometric examinations: body weight (within the accuracy of 0.1 kg), height (within the accuracy of 0.1 cm), were carried out by the established methods by means of floor weight, height meter and flexible centimeter tape. BMIs were evaluated according to standard percentile tables [5, 14]. Thus, children with BMI from 15 to 85 percentiles were assigned to have normal body mass, the excessive body mass corresponded to 85-95 percentiles and over 95 percentiles – to obesity.

To determine the factors affecting vitamin D status, the children were asked to fill in a questionnaire, which included data that ascertained the age of the child, sex, place of residence (city or village), the season of the questionnaire (November-March, April-October), income per family member (above or below the average living wage), daily milk consumption (up to 1 cup per day, from 1 to 3 cups and more), the use of vitamin D supplements, fish oil, the state of physical activity, which was determined by the number of active hours per week (up to 2 hours, from 2 to 5 hours, more than 5 hours), the duration of the daily stay in the open air (up to 30 minutes, more than 30 minutes), passive rest in front of the computer or TV (up to 2 hours per day, 2-4 hours per day, more than 4 hours per day).

Vitamin D status was determined according to the level of 25(OH)D in blood serum. For this, fasting blood test from the vein was taken. By centrifugation, serum was isolated, frozen and stored at -80 °C. The level of calcidiol was determined by the immunoassay method using 25-OH Vitamin D ELISA test kit (EUROIMMUN, Germany), with an intra-assay CV 3.2-4.9% and an inter-assay CV 4.0-7.8%. An assessment of the results of 25(OH)D level was conducted according to the recommendations of the International Society of Endocrinology (2011) [12]. Vitamin D insufficiency was established at a

level of calcidiol ranging 20-29 ng/ml (50-75 nmol/l), vitamin D deficiency was established at 25(OH)D below 20 ng/ml (less 50 nmol/l), the normal calcidiol level was at 25(OH)D 30-100 ng/ml (76-250 nmol/l). The content of 25(OH)D above 100 ng/ml (250 nmol/l) was considered to be excessive.

The attained results of the research were subjected to statistical processing. Descriptive statistics was used to evaluate the concentration of calcidiol in serum and to determine the weight-height ratios of BMI. The level of calcidiol in serum was presented in the form of mean values and their standard errors. The comparison of frequency indices in the study groups was carried out using the Wilcoxon signed-rank test for continuous variables and the chi-square test, or the Fisher's exact test for categorical variables. The comparison of mean values and their standard errors in different study groups with their accurate distribution was performed by the Student's t-test for independent samples, and if distribution of the values is not normal the nonparametric Mann–Whitney U test was used.

The multiple logistic regression was used to determine the effect of each independent variable of the probable risk factor in the development of a 25(OH)D deficiency in the adolescents with obesity. All statistical studies were conducted using SPSS (Statistical Package for Social Sciences) for Windows software 21.0 version. The differences between the values were statistically significant at $p<0.05$.

Results

The research has established low levels of 25(OH)D in serum. In the adolescents with normal body weight, the mean values of 25(OH)D were 19.76±4.28 ng/ml, in the adolescents with overweight – 15.24±3.47 ng/ml, and in the children with obesity – 13.87±2.71 ng/ml.

The results of the study of 25 (OH) D levels, depending on the body mass index, are presented in Table 1.

Vitamin D status in the adolescent children of 25(OH)D in most cases was manifested by its deficiency. In the adolescents with normal body weight, in blood serum of 14.32% of the children the level of 25(OH)D remained within the normal levels and in 29.46% was deficient.

The highest deficiency rate of vitamin D was determined in the adolescents with obesity, which prevailed with a significant difference in comparison with the incidence of vitamin D deficiency ($p=0.022$) in the control group of adolescents with normal body weight.

It has been confirmed that with the increase in BMI, a simultaneous increase in the proportion of vitamin D deficiency and a decrease in the proportion of individuals with normal levels and insufficiency of calcidiol was observed.

According to the results of statistical processing of the children's answers in the questionnaire, the frequency of manifestations of the main risk factors with underlying vitamin D deficiency in the adolescents with normal body weight, overweight and obesity has been established. The predicted risk factors for vitamin D deficiency development among the study groups, depending on the body mass index, are presented in Table 2.

Actual data have established that sex and place of residence do not have a significant impact on the prevalence of vitamin D deficiency in the adolescents with overweight and obesity. The frequency of diagnosis of vitamin D deficiency is more common in the adolescent boys with obesity, which was 42.2% ($p=0.193$). Other factors that strongly influenced the significantly greater prevalence of vitamin D were: the season of blood serum collection from November to March, low income per family member, daily milk consumption, failing to take vitamin D supplements or fish oil, low physical activity, spending much time at the computer or in front of the TV. The time spent in the open air, both with overweight ($p=0.448$) and obesity ($p=0.417$), had no effect on the incidence of vitamin D deficiency in the adolescents. For the adolescents with overweight, the duration of physical activity during the week did not influence a reliable dependence on low levels of calcidiol ($p=0.450$).

Table 1. Level of 25(OH)D in adolescents depending on the body mass index (%)

Level 25(OH)D, ng/ml	Normal body weight, (%) p=63	Excessive body weight, (%) n=68	Obesity, (%) n=78
30-100	14.32	6.75	3.83
20-29	29.46	22.61	19.17
<20	57.35	70.72	77.19*

Notes. * – significant difference between the values compare to the group with normal body weight ($p<0.05$).

Table 2. Frequency of manifestations of risk factors in the adolescents with deficiency of 25(OH)D depending on BMI % (95% CI)

Characteristics		Specific proportion of the children with deficiency 25(OH)D n the study group, % (95% CI)					
		Normal body weight, n=63	p	Excess body weight, N=68	p	Obesity, N=78	p
Sex			0.184		0.481		0.193
	men	36.1 (25.4-50.8)		40.2 (31.4-49.3)		42.2 (31.2-53.1)	
	women	25.0 (17.5-37.2)		31.3 (22.5-42.1)		35.9 (26.9-43.6)	
Place of residence			0.569		0.725		0.515
	rural areas	27.2 (18.3-36.5)		34.8 (24.1-46.2)		35.5 (24.3-46.4)	
	city	31.9 (22.7-46.3)		39.7 (27.9-48.1)		43.6 (34.9-51.7)	
Season			0.026		0.035		0.002
	April-October	18.6 (9.1-27.7)		29.4 (19.1-41.3)		26.2 (20.5-35.9)	
	November-March	41.3 (32.5-53.1)		44,1 (36.8-54.2)		52.8 (44.6- 61.1)	
Income per family member			0.019		0.032		0.006
	Above the average	15.7 (10.5-25.6)		20.5 (10.2-31.8)		28.8 (22.1-38.5)	
	Below the average	41.0 (31.6-45.9)		48.5 (36.8-61.3)		50.6 (43.4-59.5)	
Milk consumption			0.035		0.003		0.001
	Up to 1 cup per day	37.7 (26.1-44.6)		50.6 (40.2-62.5)		60.5 (51.3-69.8)	
	From 1 to 3 cups a day and more	20.8 (11.4-29.7)		20.1 (10.8-31.4)		19.8 (14.1-29.5)	
Use of vitamin D (fish oil) supplements			0.178		0.002		0.001
	yes	23.4 (12.9-32.3)		20.3 (11.8-30.9)		14.2 (7.8-23.9)	
	no	32.5 (23.8-44.2)		52.4 (42.6-67.6)		62.1 (53.8-70.2)	
Physical activity			0.198		0.450		0.001
	Up to 2 hours/week	21.2 (12.7-30.4)		30.9 (20.6-41.2)		48.7 (39.2-57.8)	
	From 2 to 5 hours per week	22.8 (14.3-31.5)		21.5 (14.7-33.8)		17,9 (11.5-25.6)	
	More than 5 hours per week	12.3 (6.3-22.8)		19.1 (11.8-32.4)		12.8 (6.4-20.5)	
Daily stay in the open air			0.251		0.484		0.417
	Up to 30 min/day	34.5 (23.8-45.3)		29.3 (20.8-38.2)		41.9 (30.6-50.1)	
	More than 30 min/day	23.0 (17.5-34.9)		41.2 (32.9-50.4)		35.5 (28.2-44.3)	
Time spent at the computer or in front of the TV			0.059		0.034		0.001
	Up to 2 hours/day	9.3 (4.8-19.2)		12.2 (6.8-22.1)		14.4 (9.3-21.8)	-
	From 2 to 4 hours/day	21.3 (12.7-33.2)		25.9 (17.5-38.4)		28.8 (23.1-36.5)	-
	More than 4 hours/day	28.2 (19.4-39.3)		35,3 (23.5-46.2)		45.4 (36.2-56.4)	

According to the results of the multiple logistic regression analysis, it has been found out that factors affecting the development of vitamin D deficiency include excessive body weight and obesity (Table 3). Moreover, in the presence of this factor, the likelihood of vitamin D deficiency increases in 1.54 times.

In addition, a significant effect on the development of vitamin D deficiency is due to winter-spring season of the study (p=0.002), low income per family member (p=0.015), low daily milk consumption (p=0.032), physical activity up to 2 hours per week (p=0.042) and more than 4 hours a day spent at the computer

Table 3. Logistic regression analysis of probable risk factors for vitamin 25(OH)D deficiencies

Risk Factor	B (SE)	OR	CI 95%	p
Sex (men versus women)	-0.14 (1,05)	0.87	0.11-6.82	0.869
Place of residence (city versus rural areas)	0.16 (0.48)	1.07	0.39-2.18	0.156
Season (November-March versus April-October)	1.29 (0.55)	2.74	1.05-7.38	0.002
Income per family member (below the average versus above the average)	2.08 (1.17)	1.31	0.52-6,14	0.015
Milk consumption (up to 3 cups or more versus up to 1 cup)	-1.54 (0.95)	0.67	0.24-0.93	0.032
The use of vitamin D supplements (fish oil) (no versus yes)	0.91 (1.07)	1.46	0.31-5.79	0.698
Physical activity				
Up to 2 hours/week versus more than 5 hours/week	1.36 (0.42)	1.61	0.83-3.45	0.042
2 to 5 hours/week versus more than 5 hours/week	0.48 (0.76)	1.01	0.45-2.15	0.253
Daily stay outdoors				
Up to 30 minutes/day versus more than 30 minutes/day	-0.72 (0.93)	0.89	0.24-2.09	0.062
Time spent at the computer or in front of the TV				
2 to 4 hours/day versus 2 hours/day	0.32 (0.83)	1.27	0.28-7.03	0.720
More than 4 hours/day versus 2 hours/day	0.27 (0.69)	1.91	0.35-8.46	0.027
Excessive weight, obesity	0.43 (0.85)	1.54	0.37-3.02	0.012

or TV (p=0.027). Along with this, it has been found out that sex (p = 0.869), place of residence (p=0.156), taking of vitamin D supplements, fish oil (p = 0.698), daily outdoor exposure (p=0.062) have no significant effect on the development of vitamin D deficiency in the children with overweight and obesity.

Discussion

The results of the study have proved that the prevalence of vitamin D deficiency in the adolescents is significant as in many other countries [2, 3, 12]. It has been established that there is an inverse relationship between the level of 25(OH)D in blood serum and the body mass index in the adolescents. In cases of excessive body weight, the frequency of diagnosing vitamin D deficiency increased in 1.23 times, and with obesity – by 1.35 times. The mean serum calcidiol content in blood serum of the adolescents with obesity was 1.43 times lower than that of the children with normal body weight. The data attained during the study showed a similar trend of change in the status of vitamin D in the children of different ages according to the results of epidemiological studies in Ukraine but were lower compared with the data of the studies in the USA, Spain, and Italy [5, 9]. Researchers explain the low levels of 25 (OH) D in blood serum by depositing calcidiol in the adipose tissue, reducing bioavailability, and reducing its synthesis under the influence of ultraviolet rays [14].

According to the results of the conducted studies, it has been established that the prevalence of vitamin D deficiency in the adolescents with obesity and overweight is unrelated to sex and place of residence. The latter were also not recognized as probable risk factors for vitamin D deficiency. However, according to Spanish pediatric school [5], vitamin D deficiency was more often reported during puberty in obese girls.

Via the multiple logistic regression analysis, it has been established that the degree of influence of independent predictors do affect development of vitamin D deficiency in the adolescents with obesity and overweight. It has been proved that the greatest influence is exerted by the season of blood collection in the period of November-March, in which the probability of development of vitamin D deficiency increases in 2.74 times compared with the April-October season. The amount of time spent at the computer and watching TV more than 4 hours a day increases the chances of vitamin D deficiency development in 1.91 times and, together with low physical activity, belongs to the three main independent variables in the development of vitamin D deficiency in the adolescents with obesity and overweight. Research results also indicate that the daily milk consumption of up to 3 cups or more reduces development of vitamin D deficiency in 1.49 times compared with the adolescents, who do not consume or consume

up to 1 cup of milk per day. Our data support the results of studies conducted by the scientists from other countries [8, 10] concerning the degree of insufficiency or deficiency of vitamin D caused by the above-mentioned risk factors.

For a comparative assessment of the impact of poverty and the level of income per family members on vitamin D status, we included in the questionnaire the information about the income of the adolescent's family. It has been established that the level of low income per family member increases in 1.31 times the likelihood of vitamin deficiency in adolescents ($p = 0.015$). The findings confirm the results of other studies conducted in different countries, but in that case, the risk ratio was 1.36, while in the USA it was 1.6 [13], and in Canada – 3.14 [14].

On the other hand, we have not confirmed the significance of vitamin D supplements and fish oil as a factor for vitamin D deficiency. In our opinion, it is mainly due to the low amount of food and milk products enriched with vitamin D or their use in insufficient quantities, as well as irregular use of fish oil.

Consequently, according to the results of the conducted studies, the prevalence of vitamin D deficiency and factors of its development in the children with overweight and obesity have been defined as well as the main probable factors of its development.

Conclusions

Vitamin D deficiency is prevalent in adolescents with overweight and obesity. The main risk factors for vitamin D deficiency development include winter and spring seasons, spending more than 4 hours per day at the computer, low physical activity up to 2 hours per week, taking small portions of milk less than 1 cup per day and low income per family member. To prevent development of hypovitaminosis and vitamin D deficiency, it is necessary to carry out educational activities with adolescents aimed at healthy lifestyle and healthy eating, and to develop an optimal program for improving vitamin D status in obese children.

ПОШИРЕНІСТЬ ТА ФАКТОРИ РИЗИКУ РОЗВИТКУ ДЕФІЦИТУ ВІТАМІНУ Д У ПІДЛІТКІВ З НАДМІРНОЮ МАСОЮ ТІЛА ТА ОЖИРІННЯМ

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

Вступ. *Вітамін Д бере участь у функціонуванні багатьох органів і систем організму. Одним з факторів ризику розвитку гіповітамінозу Д є порушення обміну ліпідів, і кількість жирової тканини відіграє вирішальну роль за даних обставин.*

Метою дослідження *стало вивчення поширеності та факторів ризику розвитку недостатності вітаміну Д серед підлітків з надмірною масою тіла та ожирінням.*

Методи дослідження. *Обстежено 146 дітей з надмірною масою тіла та різним ступенем ожиріння та 63 здорових дітей з нормальною вагою. Усі включені у дослідження підлітки не вживали препарати вітаміну Д. Для оцінки стану забезпеченості організму визначали рівень кальцидіолу 25(OH)D у сироватці крові. Недостатність вітаміну Д діагностували при значеннях показника 20-29 нг/мл, а його дефіцит – при рівні менше 20 нг/мл. Нормальний вміст кальцидіолу коливається в межах 30-100 нг/мл.*

Результати. *Середній рівень 25(OH)D у підлітків з нормальною масою тіла склав (19,76±4,28) нг/мл, з надмірною масою тіла – (15,24±3,47) нг/мл, з ожирінням – (13,87±2,71) нг/мл. Поширеність дефіциту вітаміну Д у дітей з надмірною масою тіла склала 70,62 %, з ожирінням – 77,19 %.*

Висновки. *Дефіцит вітаміну Д переважає серед підлітків з надмірною масою тіла та ожирінням. Для профілактики розвитку його недостатності необхідно пропагувати здоровий спосіб життя, заохочувати фізичну активність та здоровий спосіб харчування та розробити оптимальні програми для покращення ситуації серед дітей з ожирінням.*

КЛЮЧОВІ СЛОВА: **вітамін Д; діти; кальцидіол; поширеність; ожиріння.**

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MORPHOLOGICAL RESPONSE TO IMPLANTATION OF A POLYPROPYLENE MESH WITH A PRF MEMBRANE IN PATIENTS WITH POSTOPERATIVE VENTRAL HERNIA AND UNDIFFERENTIATED CONNECTIVE TISSUE DYSPLASIA

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Background. *Current herniology promotes the widespread usage of mesh implants in the primary treatment and, especially, in the for postoperative ventral hernias.*

Objective. *The aim of the research was to study the morphological response of the tissues of muscular aponeurotic layer to implantation of a polypropylene mesh with using PRF membrane-enriched platelets in the patients with postoperative ventral hernia and concomitant undifferentiated dysplasia of connective tissues.*

Methods. *The research involved 98 patients with postoperative ventral hernia, who underwent retro-muscular alogernioplasty by the Sublay technique of implantation of 'light' meshes, and a 'light' polypropylene mesh (PPM) in combination with a platelet-rich fibrin (PRF) membrane. The patients were divided into experimental groups according to the presence of undifferentiated connective tissue dysplasia syndrome.*

Results. *Microscopic studies carried out after the implantation of a polypropylene mesh with a PRF membrane has proved that structural changes in connective tissues are like those of a polypropylene mesh, but they are less significant. There was a leukocyte infiltration near the mesh material, but its area was small. The enlargement and blood filling of the vessels of microcirculatory channel was a manifestation of the increased vascularization of this area.*

Conclusions. *The usage of a polypropylene mesh in combination with a PRF membrane in the surgical treatment of postoperative ventral hernias reduces inflammatory changes in the tissues significantly and increases the activation of fibroblasts and signs of collagen fibers around the mesh material that is relevant especially for the patients with connective tissue pathology.*

KEY WORDS: **postoperative ventral hernia; undifferentiated connective tissue dysplasia; polypropylene mesh; PRF membrane.**

Introduction

Contemporary herniology promotes the widespread usage of mesh implants in the primary treatment and, especially, in the postoperative period of ventral hernias. This is supported by the improved methods of surgical treatment and high-quality allotransplants. However, alologernioplasty does not always provides reliability of the surgery [1, 2, 3]. Adequately selected techniques and materials for aloplasty minimize surgical tactical and technical causes of the development of relapse [4]. Therefore, the identification of other objective causes remains relevant. One of these conditions, which can cause a rapid relapse, is a concomitant syndrome of undifferentiated connective tissue dysplasia (UCTD). Such surgical intervention using mesh implants does not always allow achieving the expected result [5].

In our opinion, the study of combined usage of polypropylene mesh with a PRF membrane consisting of fibrin-rich platelets is very interesting [6, 7]. Biocompatible PRF membranes rich in growth factors stimulate an active growth of new capillaries, improve blood flow, accelerate metabolic processes in tissues, increase collagen formation, hyaluronic acid, reduce inflammatory process in tissues significantly that can positively affect the state of local tissues during implantation of polypropylene meshes in cases of UCTD.

The aim of the research was to study peculiarities of morphological response of the tissues of muscular aponeurotic layer of anterior abdominal wall to implantation of polypropylene mesh with a PRF membrane in the patients with concomitant undifferentiated dysplasia of connective tissues.

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Methods

98 patients underwent surgery at the premises of the Department of Surgery of Ternopil City Clinical Hospital No. 2 in the period from 2015 to 2017, in the Department of Surgery of the Institute of Postgraduate Education of I. Horbachevsky Ternopil State Medical University. The study involved patients with postoperative ventral hernias. All patients underwent retro-muscular alogernioplasty by the Sublay technique of implantation of a 'light' mesh, the diameter of polypropylene thread 0.12 mm, the thickness 0.40 mm, the specific density 45 g/m^2, and an 'light' polypropylene mesh (PPM) in combination with a platelet-rich fibrin (PRF) membrane. The patients were divided into experimental groups according to the presence of UCTD syndrome

The diagnosis of UCTD syndrome was based on specific phenotypic features of dysplasia (6 and more) according to the international M. J. Glesby phenotypic scale and biochemical markers of connective tissue degradation – a serum oxyproline level (L. Bergman and R. Loxley colorimetric method modifications by M. A. Osadchuk and T. P. Kuznetsova), and cryoglobulins (by the method of N. A. Konstantinova and A. Yu. Kirsanov, 1989). For morphological study of diagnosis of non-specific dysplasia of connective tissue, during the surgery a sampling of the aponeurosis fragments of white abdominal line was carried out near the hernia defect of 0.3×0.3 cm in size. The fragments of anterior abdominal wall with the implanted mesh were taken in 12 patients for further morphological study following the norms of medical bioethics; the patients were re-operated for other pathology of abdominal cavity.

The tissues were fixed in formalin, paraffin embedded and sectioned according to the standard technique. Serial 5 μm sections were stained with hematoxylin & eosin, blood-tyrosine and examined by light microscopy using Delta Optical microscope. Representative areas of the samples were photographed using ×10 and ×20 lens with SCMOS Digital Camera and ToupView software with different magnification.

Results

According to the specific phenotypic features of dysplasia and biochemical markers of connective tissue degradation, all patients were divided into 2 groups: with and without UCTD syndrome (Table 1).

Histological studies performed after 'light' PPM implantation have proved that the changes in inflammatory nature are observed around the structure of the mesh. There are lymphocytes, neutrophils, macrophages and separate basophils in the area of leukocyte infiltration. The modified fibroblasts and fibrocytes are present. There are a few fibrous structures that are loose and partially fissured in the intercellular substance. The intensified vasculitis of this site, many vessels of the microcirculatory bed, which are blood-filled (Fig. 1), are evidenced.

The results of histological studies of the area of white abdominal line near the hernial protrusion in the patients with undifferentiated

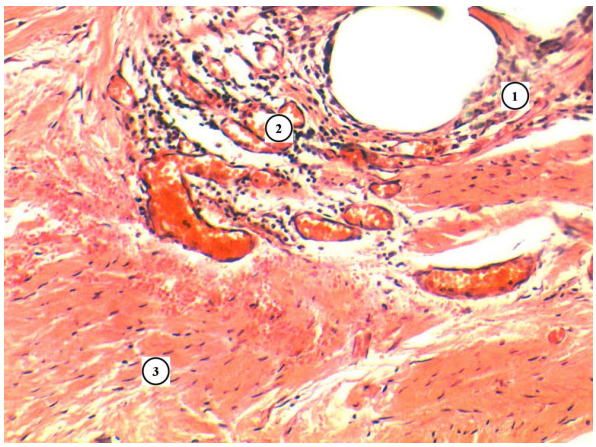

Fig. 1. Microscopic changes in the connective tissues surrounding the implanted material – the polypropylene mesh. Infiltrated area (1), blood vessels (2), muscle tissue (3). H&E. ×100.

Table 1. Age, sex and type of the implanted mesh division of the examined patients (M±m)

Investigated groups	Types of the implanted mesh		Patients' age	Patients' sex	
	'Light' PPM	'Light' PPM + PRF membrane		Male	Female
Group 1 (n=53) without UCTD	28	25	52.2±0.89	18 (33.96%)	35 (66.04%)
Group 2 (n=45) with UCTD	26	19	37.4±1.12	21 (46.67%)	24 (53.33%)

dysplasia of connective tissues have revealed that the collagen and elastic fibers are thinned, with a fine mesh, in different directions and in different spaces. Branching of fibers is evidenced and individual fibers completely lose contact with the main fibers (Fig. 2).

Moderate lymphostiocytic infiltration around the fibers has been observed according to the results of histological study of the area of muscle fibers with a segment of the 'light' polypropylene mesh, which is combined with severe stroma edema, folding of collagen fibers, moderate mucoid edema. There is a mild vascular reaction in the form of a full-bladder capillary combined with perivascular cell infiltration. The swelling spreads to muscle tissues, accompanied by inflammatory infiltration, destruction of fibers and hemorrhagic penetration of tissues (Fig. 3).

Microscopic studies carried out after the implantation of the polypropylene mesh with a PRF membrane have proved that structural changes in connective tissues are similar to those of the polypropylene mesh, but they are less significant. There is leukocyte infiltration near the mesh material, but its space is small. The enlargement and blood filling of the vessels of microcirculatory channel, which is a manifestation of the increased vascularization of this area, is evidenced (Fig. 4).

The formation and concentric arrangement of collagen fibers around the grid structures is revealed. This takes place with the participation of mature fibroblasts. The fragments of mesh fibers are surrounded by collages of collagen fibers, concentrically around the grid structures and arranged at a certain distance. There is a longitudinal shape between fibers with thin fibroblasts, oriented in the direction of the fibers (Fig. 5).

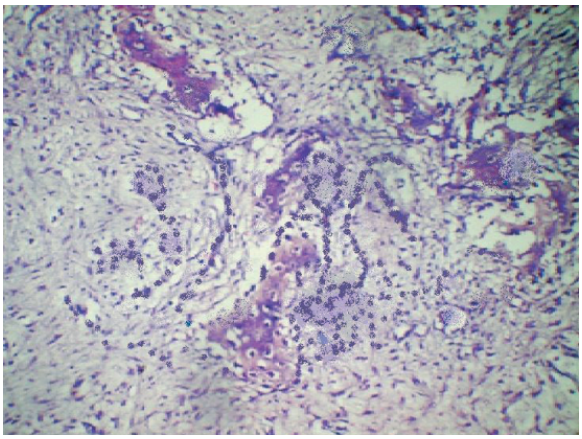

Fig 2. White line of abdomen near the hernial protrusion and UCTD. Blood-tyrosine. ×100.

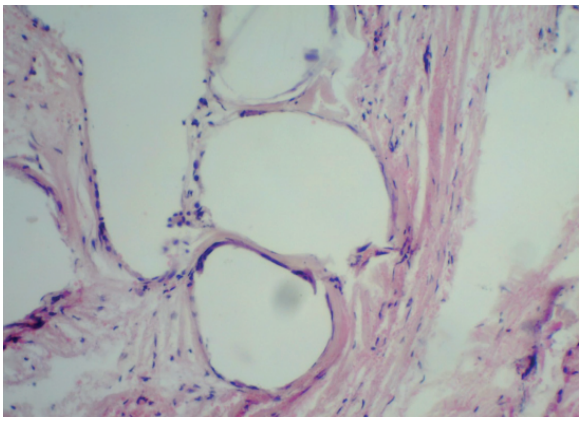

Fig. 3. The area of muscle fiber with a fragment of a polypropylene mesh. Destruction of fibers and hemorrhagic penetration of tissues. H&E. ×100.

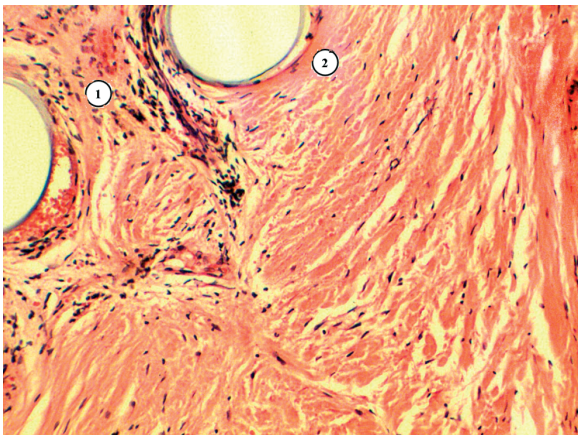

Fig 4. Microscopic changes of the connective tissue surrounding the implanted material – a 'light' polypropylene mesh with a PRF membrane. Infiltrated area (1), collagen fibers (2). H&E. ×100.

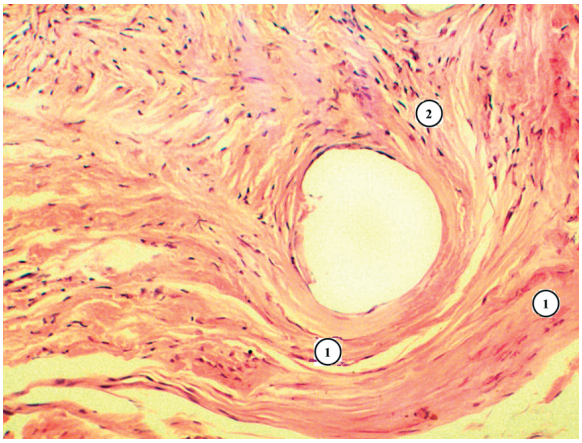

Fig 5. Microscopic changes of the connective tissue of the area surrounding the implanted material – a polypropylene mesh with a PRF membrane. Collagen fibers are located concentrically (1), fibroblasts (2). H&E. ×100.

Discussion

Recurrence after hernia surgery is a considerable clinical problem. Surgical treatment of ventral hernia accounts a hundred methods for today. It means we are still looking for better outcomes and results in postoperative period. The most wanted outcome of surgical treatment of ventral hernias is the absence of relapse for many years. But no one method could provide it today for the results of long prospective observational studies.

Family history, biochemical factors, smoking, method of repair, concomitant bowel surgery, gender, obesity and other factors are very important for hernia recurrence. The research confirms that undifferentiated connective tissue dysplasia is significant factor for abdominal hernia formation [4, 7, 8, 9]. Thus, microscopic studies after the implantation of the polypropylene mesh in combination with a PRF membrane have revealed that inflammatory changes in connective tissues with underlying dysplasia are not as significant as in cases of implantation of the polypropylene mesh only. The activation of fibroblasts and signs of formation of fibrous structures around the mesh material have been revealed. This contributes to increased activity of fibroblasts and formation of collagen fibers around the mesh material.

Syndrome of undifferentiated connective tissue dysplasia quite often is masked at common surgical nosology. Its frequency varies and is diagnosed at approximately in one-third of patients of surgical departments. Just because surgeons do not take into account this syndrome mostly, it became the cause of postoperative relapse in each second patient with ventral hernias [9, 10]. Results of our previous research and histological, morphometric examination, immunological tests of patients underwent the surgery with use of 'light' polypropylene mesh in combination with a platelet-rich fibrin (PRF) membrane showed significantly better outcomes. The rate of recurrences in patients with postoperative ventral hernia, who underwent retro-muscular alogernioplasty was significantly lower [10-12].

Conclusions

The usage of a polypropylene mesh in combination with a PRF membrane reduces inflammatory tissue changes significantly, increases activation of fibroblasts and signs of collagen fibers around the mesh material, especially in the patients with connective tissue pathology.

The use of PRF membranes of blood plasma stimulates angiogenesis, improves blood flow, accelerates metabolic processes in tissues, formation of collagen that creates favorable conditions for a full integration of the polypropylene mesh into the muscular and aponeurotic layer of the tissues of the anterior abdominal wall, as a result it contributes to reduction of postoperative relapse.

МОРФОЛОГІЧНА ВІДПОВІДЬ НА ІМПЛАНТАЦІЮ ПОЛІПРОПІЛЕНОВОЇ СІТКИ З МЕМБРАНОЮ PRF У ХВОРИХ З ПІСЛЯОПЕРАЦІЙНИМИ ВЕНТРАЛЬНИМИ ГРИЖАМИ І НЕДИФЕРЕНЦІЙОВАНОЮ ДИСПЛАЗІЄЮ СПОЛУЧНОЇ ТКАНИНИ

В. І. Пятночка, А. М. Продан

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

Вступ. *Сітчасті (mesh) імплантати сьогодні широко використовуються для первинного лікування вентральних гриж, а особливо у випадку розвитку післяопераційних гриж.*

Метою дослідження *стало вивчення морфологічної реакції тканин м'язового апоневротичного шару на імплантацію поліпропіленової сітки з PRF-мембраною, збагаченою тромбоцитами, у пацієнтів з післяопераційними вентральними грижами та супутньою недиференційованою дисплазією сполучних тканин.*

Методи дослідження. *У дослідженні брали участь 98 пацієнтів з післяопераційними вентральними грижами, яким проведено герніопластику власнетканинну за типом "sublay" за методикою імплантації «легких» сіток і «легкої» поліпропіленової сітки (PPM) у поєднанні з PRF-мембраною, збагаченою тромбоцитами. Пацієнти були розділені на групи залежно від наявності недиференційованого синдрому дисплазії сполучної тканини.*

Результати. *Мікроскопічні дослідження, проведені після імплантації поліпропіленової сітки з PRF мембраною, показали, що в сполучних тканинах розвивалися структурні зміни подібні до таких, як при і при пересадці лише поліпропіленової сітки, але вони були значно менш виражені. Біля сітчастого матеріалу подекуди було зафіксовано інфільтрацію лейкоцитами, але її площа була невеликою. Збільшення і кровонаповнення судин мікроциркуляторного каналу було проявом підвищеної васкуляризації цієї ділянки.*

Висновки. *Використання поліпропіленової сітки у поєднанні з мембраною PRF в хірургічному лікуванні післяопераційних вентральних гриж значно знижує запальні зміни в тканинах і збільшує активацію фібробластів і розвиток колагенових волокон навколо сітчастого матеріалу, що особливо актуально для пацієнтів з патологією сполучної тканини.*

КЛЮЧОВІ СЛОВА: **післяопераційна вентральна грижа; недиференційована дисплазія сполучної тканини; поліпропіленова сітка; мембрана PRF.**

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RECONSTRUCTIVE SURGERY OF SEVERE DAMAGES OF LOWER EXTREMITIES INTEGUMENT AFTER INJURY

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Background. *Injury of lower extremities requires the fastest and most effective method of closing wound surfaces.*

Objective. *The aim of the study was to define the indications and improve the reconstructive interventions for severe damage of lower extremities integument due to mechanical trauma.*

Methods. *During 2008-2016, 242 patients with defects of cover tissues of the body and extremities were operated in the clinic. Depending on the size, depth and degree of tissues damage, all were divided into 4 groups.*

Results. *The lower extremity was the most vulnerable segment (75.2% of patients). Damage Control tactics was used in 83% of cases. To protect the functionally tense areas, free plastics by a split skin graft (the 1st group – 12.8%, the 2nd – 20.4%, the 3rd – 37%, the 4th – 8.9%) were used for closure of the defect. If the wound defect affected functionally significant structures up to 1% of the body surface, the complex flaps of local tissues, tissues close to the defect and anatomically distant areas (the 2nd group – 6%, the 3rd – 10.6%, the 4th – 4.3%) were used. If the defect was more than 1% of the body surface only functionally tense areas were closed with compound complexes of tissues. The rest of the skin was restored by means of autodermoplastics. We suggested and approved our specific protocol of treatment of such injury.*

Conclusions. *Implementation of the suggested protocol of reconstructive interventions for closure of the defects of cover tissues of lower limbs allowed attaining a positive result in 98.8% of the interventions.*

KEY WORDS: **trauma; soft tissue defect; wound surface; flaps; lower extremities.**

Introduction

The defects of cover tissues caused by mechanical factors differ regarding major anatomical and functional changes, damage to main vessels and nerves, bleeding, fractures of partial or complete separation of limb segments [5, 6, 7]. Injury is always accompanied by ischemia and infection of soft tissues and therefore it requires the fastest and most effective method of closing wound surfaces [2, 3, 4, 9, 14]. The nature of the damage in acute trauma of lower limbs, on the one hand depends on the weight, speed, direction and duration of the traumatic agent, on the other – on the location, anatomical and physiological characteristics of the damaged structures [1, 10, 11, 15]. All the above requires a careful evaluation of the volume of reconstruction to restore the form and function of a lower limb [8, 12, 13, 16].

The aim of the study is to define the indications and improve the restorative reconstructive interventions in cases of severe damage of cover tissues of lower extremities of the mechanical genesis.

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Methods

242 patients were involved into study. All of them had defects of cover tissues of the body and extremities as a result of mechanical damage were operated in the Regional Centre of Plastic and Reconstructive surgery (Zaporizhzhia, Ukraine) for the period of 2008-2016. The lower extremities were damaged in 182 cases (182/242; 75.2% of patients). Most of the surgical interventions were performed for tissue restoration: 334 among all 472 (334/472, 70.8%), 235 were reconstructions. The criteria for inclusion in the study were: age of over 17 years old of both sexes with a diagnosed defect of skin and underlying soft tissues of the body and limbs, requiring restoration of form and function of the body. The criteria for excluding

from the study were: patients age of below 17 years old, defects of face and head, ulcerative defects that were developed due to chronic vascular or neurological pathology, as well as the consequences of purulent inflammatory diseases or malignant neoplasms of skin.

Depending on the size, depth and degree of damage of the tissues of lower extremities, all patients were divided into 4 groups. The 1st group I involved 31 (31/182; 17%) patients with a narrow (up to 5 cm in diameter) area of damage of skin and underlying tissues to deep fascia. The 2nd group counted in 56 (56/182; 30.8%) patients with large and extra-large wound surface and damage to soft tissues below deep fascia. The 3rd group was composed 75 (75/182; 41.2%) patients with defects of cover tissues, which developed together or as a result of damage of the osteoarticular apparatus. The 4th group involved 20 (20/182; 11%) patients with combined or multiple trauma accompanied by damage to major vessels, nerves, partial or complete secretion of a limb.

In the 1st group of there were 13 (13/31; 41.9%) males, 18 (18/31, 58.1%) females; average age – 53 years old. In the 2nd group there were 20 (20/56; 35.7%) males, 36 (36/56, 64.3%) females; the average age was 54 years old. In the 3rd group there were 56 (56/75; 74.7%) males, 19 (19/75, 25.3%) females, the average age was 51 years old. In the 4th group there were 17 (17/20; 85%) males, 3 (3/20, 15%) females, the average age – 48 years old.

In all four groups (the 1st, 2nd, 3rd, 4th), there were 76 (41.8%) females and 106 (58.2%) males in the study, the average patients age was 51.8 years old.

All patients were examined by standard clinical and laboratory methods, which included blood and urine tests, total protein and its fractions, glucose test, electrolytes and acids, basic-acid balance, bilirubin, coagulation profile, creatinine, urea, amylase and aminotransferases (ALAT, ASAT) activity, microbiological and cytological examination of the wounds.

Duplex ultrasound (DU) results were one of the most important diagnostic criteria for choosing the method of surgical intervention: marking the feeding vessel of the future complex flap on axial or segmental blood supply. In that case, the length and diameter of the vessel, the depth of its occurrence, the presence of perforations and collateral branches were evaluated. The study was carried out using the Vivid 3 Expert device General Electric (USA) by a linear sensor with a frequency of 5 MHz. The method allowed visualization of the arteries and veins in real time, studying the functional parameters of blood flow and the condition of surrounding tissues. A comparative assessment of the indicators with those of the opposite limb was performed to reveal a blood flow deficit or severe violations of blood flow. Also, DU allows to investigate the regional hemodynamics was in the injury area and in the donor area of the future non-free complex flap. The quantity of DU: in the 1st group 1 examination was performed, in the 2nd group – 12, in the 3rd – 11, in the 4th – 12.

Results

The choice of the reconstruction method for repair of the defects of cover tissues depended on the volume of damage, as well as on anatomical and functional characteristics and hemodynamic features of the traumatized area.

In 1st group 38 (38/472; 8.1 %) interventions were performed, 8 of which were primary surgical treatment of the wound at the hospitalization stage, and 30 (30/334; 9%) – skin restoration interventions.

The 2nd group patients underwent 126 (126/472, 26.4%) surgeries, 29 – a primary surgical treatment of wounds, autopsy and drainage of hematomas, 97 (97/334; 29 %) – interventions for closure of cover tissue defects.

In 3rd group 237 (237/472, 50.2%) operations were performed, 28 of them were primary surgical treatment of wounds, 4 – opening and drainage of hematomas, 1 – fasciotomy, 40 – operations for bone restoration, 1 – thoracoscopy, 4 – laparocentesis; 159 (159/334; 47.6%) – operations for closure of wound surfaces and defects of cover tissues.

In 4th group, 71 (71/477; 14.9%) patients underwent interventions at the first stage for initial debridement (6 cases), 2 – for drainage of hematomas and wounds treatment, 5 – for fasciotomy, 10 – for bone restoration. In one case, laparocentesis and drainage of abdominal cavity were performed.

48 interventions among 339 (48/334; 14.4%) were performed for restoration of vessels, nerves, and closure of wound defects.

Depending on the depth and mechanism of the injury, combined or multiple trauma, concomitant illnesses, the following surgical interventions for restoration of cover tissues were performed. Free split skin graft was used for the patients of all groups (the 1st – 30/235; 12.8 %, the 2nd – 48/235, 20.4%, the 3rd – 87/235, 37%, the 4th – 21/235, 8.9%) with superficial

granulating wounds of different sizes. One or several grafts were received by disk dermatoms DED-75 (Russia) and DD-717 (Ukraine) or a razor blade, depending on the size of the wound. The thickness of the graft varied from 0.25 to 0.6 mm.

If size of the wound did not exceed 1% (patient's palm size), the method of autodermoplasty by Tyrsh was used, when one or more grafts were taken using a disposable razor blade (thickness of the layer 0.25-0.3 mm).

With proper taking of the graft (thickness of the graft did not exceed two thirds of the derma), the healing processes of the donor's zone was without any functional and aesthetic deficiency. In all groups (the 1st, 2nd, 3rd, 4th), the patients experienced no complications in healing of the donor area.

At the stage of preparation for the surgery, the necrotic tissues were cleaned out of the wounds, marginal cavities were removed, as well as additional hematoma drainage. Stage necrotomy was performed surgically or using bandages, creams, ointments to treat wound and control the microflora. The transplantation was carried out using the standard method. The autodermotransplant was laid out on the wound surface. With two or more transplants, their edges were carefully compared, fixed with individual seams along the edges if necessary (Fig. 1 A, B).

In 2 (2/235; 0.9%) cases (1 in the 2nd group and 1 in the 3rd group) there was a partial lysis of autodermotransplants requiring additional surgical interventions. There were no complications after repeated operations.

By transplantation of free split skin grafts, the surface-granulating wounds of different in size were closed as quickly as possible. This was especially important in the patients with multiple and combined traumas requiring multistage interventions into various anatomical structures. The main disadvantage of this technique was the formation of a structurally and aesthetically defective skin cover in the area of damage. The most optimal result of the study was attained by using different types of flaps with a nourishing stalk.

The indications for use of complex flaps in plastics were:

– the damaged area was a functionally tense part of the limb (projection of joints) or a subject to high mechanical stress (heel, sole);

– poor blood supply to the defect area and the surrounding cover tissues;

– depth of the defect, at the bottom of the wound a freely settled bone, joint, tendon, vessel, nerve;

– elimination of contour defects of the limbs.

The choice of the donor area depended on a patient's age, concomitant pathology, features of regional hemodynamics in the region of the injury. The main principle was a distance to the damage zone – closer to the defect, the better the result of surgical intervention was. An important issue was the scarring in the donor area not to cause functional disorders and have a minimal aesthetic deficit.

The flap with a nourishing stalk can be cut out of the cover tissues that are directly adjacent to the defect, the tissues that are close to the defect or distant anatomical zones (Fig. 2 A, B).

In the 1st group (n=31), the patients with a narrow (up to 5 cm in diameter) area of da-

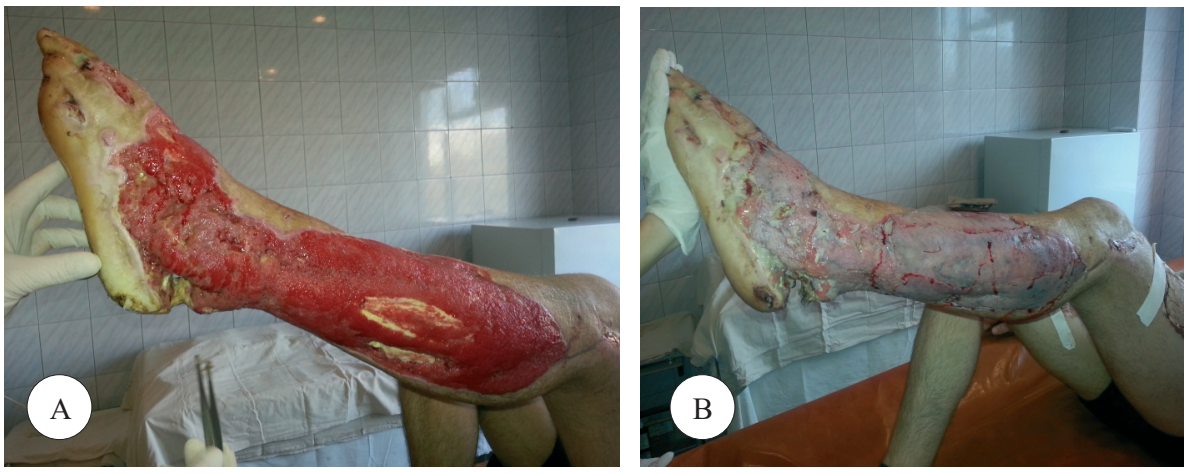

Fig. 1 A, B. Plastics with a free split skin transplant of a semicircular post-traumatic defect of the left shin (patient D., Case No 14318).

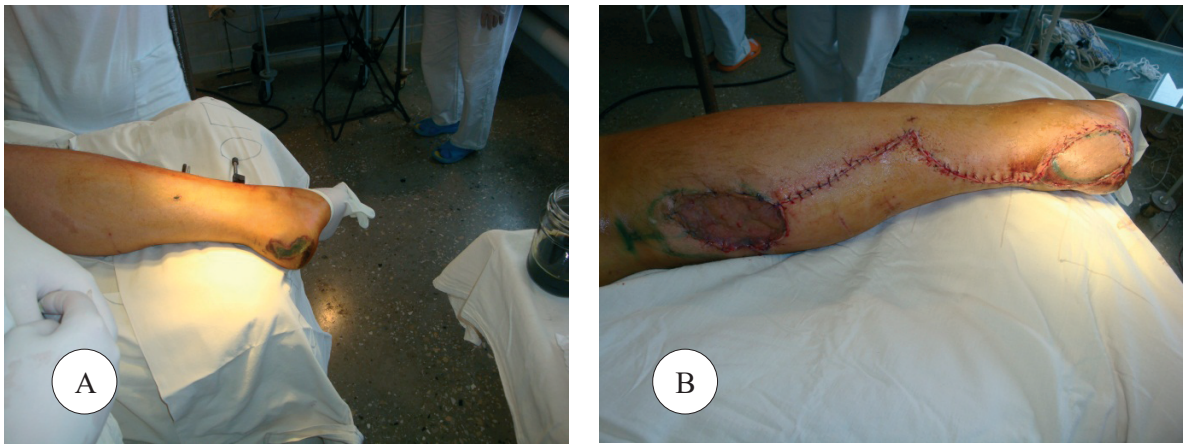

Fig. 2 A, B. Plastic wound defect with five islet flaps with a nourishing stalk (S. S., Case No 20414).

maged skin and underlying tissues to deep fascia, the flap with a nourishing stalk was not used because of the lack of indications for reconstructive interventions.

The patients of the 2nd group (n=56) with a large and extra-large wound area and damage of soft tissue to deep fascia underwent 8 plastics of tissues around the defect using flaps, 5 of which were local plastics by means of flat, sliding flaps, and 3 – by transposition flaps. 6 surgical interventions were performed by flaps of the tissues adjacent to the defect of anatomical sites: 1 – an islet flap with a peripheral stalk, 2 – a bridge-like flaps (a flap with two nourishing stalks), 1 plastic by a flat flap using the technique of derma tension (3 surgical interventions). Totally 14 surgical interventions with complex flaps were performed.

In the 3rd group (n=75), 25 surgical interventions using the flaps with nourishing stalks were performed for the patients with defects of cover tissues that developed together or as a result of damage of osteoarticular apparatus. 3 plastics by local tissues were conducted: 2 – by a sliding flap, 1 – by a transposition flap. 2 surgical interventions were performed by a flap of the tissues adjacent to the defect of anatomical sites: 1 – by an islet flap with a peripheral stalk, 1 plastic – using a flat flap. 20 operations were performed by means of a tubular migratory classical flap (of a remote anatomical site).

The patients of the 4th group (n=20) with combined or multiple trauma accompanied by damage of main vessels, nerves, partial or complete separation of a limb underwent 10 reconstructions using flaps with a nourishing stalk. 7 plastics were performed using the tissues around the defect: 6 – by sliding flaps, 1 – by a transposition flap. 1 closure of the wound defect was conducted by an isled flap on a peripheral stalk, 2 interferences were conducted by flat flaps, which were formed using the technique of tissue derma tension.

In 1 (1/235; 0.4%) case (in the 3rd group) there was a partial ischemic necrosis of the flap, which required additional surgical interventions. No complications after a repeated operation were present.

Discussion

Only in 17% (the 1st group, n=31/182) of the patients the lower limb trauma was an isolated damage of cover tissues with limited (up to 5 cm in diameter) area of the skin and subordinate tissues damage to deep fascia.

In 83% of patients (the 2nd, 3rd, 4th groups, n=151/182), the injuries were caused by a high-energy trauma, in 41.2% (the 3rd group, n=75/182), the defects of cover tissues developed together or as a result of damage of osteoarticular apparatus, 11% (the 4th group, n=20/182) of patients suffered from combined or multiple injuries that were accompanied by damage to great vessels, nerves, partial or complete abruption of a limb. Therefore, the treatment of the patients of the 2nd, 3rd, 4th groups involved mainly the Damage Control tactics, the main principles of which were to support or sustain life, minimize additional injuries from primary intervention and perform the final surgical reconstruction of a damaged area after stabilizing the patient's condition (Fig. 3).

In cases of initial cover tissues defect of a lower limb, the choice of reconstruction technique depended, according to the algorithm, on the severity of patient's condition on admission. Thus, the patients in a stable condition

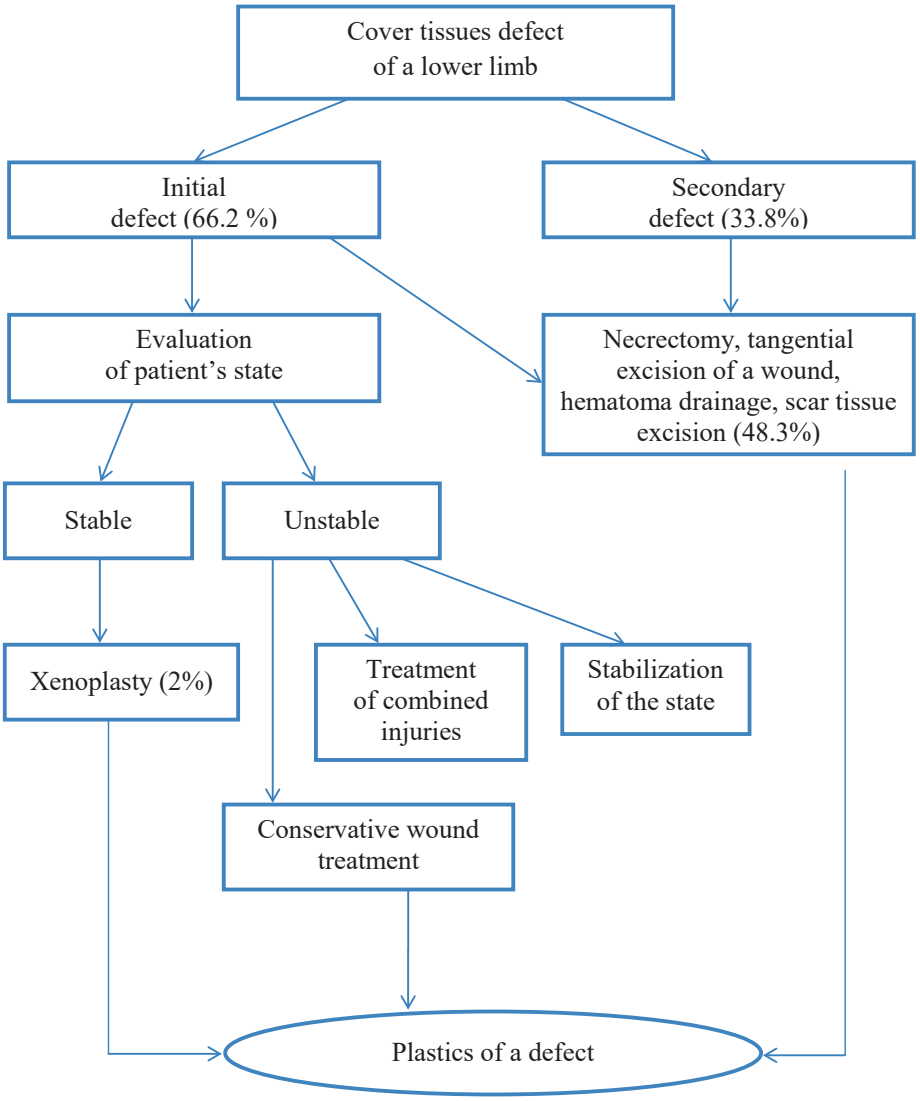

Fig. 3. Algorithm of surgical treatment of cover tissues defect of a lower limb

underwent a primary surgical treatment of the wound according to the standard method; 2% of the patients underwent xenoplasty for temporary closure of the wound surface with the subsequent restoration of cover tissues.

If the patient's condition was unstable, after intensive therapy, normalization of hemodynamics, primary surgical treatment of the wound, stabilization of fracture, management of limb ischemia, the reconstruction of cover tissues was delayed.

The choice of the corresponding tissue graft depended on localization, size of the defect and volume of the damage of subordinate soft tissue structures (Table 1).

Thus, the primary cover tissues defect of a lower limb was present in 100 (66.2%) patients: in the 2nd group – 46 patients, in the 3rd group – 44, in the 4th group – 10 patients. In cases of localization of the wound surface not in the projection of functionally tense areas, i.e. hip, knee, ankle-foot joints, for the closure of the defect, a free plastic by a split skin graft was used

If the wound defect involved functionally significant structures such as exposed bones and tendons, joint areas and had an area of up to 1% of the body surface, the flaps out of local tissues and tissues close to the defect were used. In 2 patients with cover tissues defect of a limb stump, and in 1 – of a foot stump, the flaps out of anatomically distant areas (tubular flaps) were used to form a full-value stump under the prosthesis. If the defect was more than 1% of the body surface, only functionally tense areas were closed with compound complexes of tissues. The rest of the skin was restored by means of autodermoplastics.

Table 1. Characteristic features of the tissues used for closure of the cover tissues defect of a lower limb

Tissues composition	Foot				Shin				Hip			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Free skin graft (n)	6	3	19	4	24	36	43	7	1	5	5	
Local tissues (n)	-	1	1	-	-	6	2	5		2	1	1
Tissues close to the defect (n)	-	-	1	-	-	2	-	1		1		2
Compound complexes out of distant areas (n)	-	-	1	-	-	-	2	-	-	-	-	-

Notes: n – number of patients.

There were twice less patients with secondary cover tissues defects – 51 (33.8%) patients: 10 patients in the 2nd group, 31 in the 3rd group, 10 patients in the 4th group.

These cases were associated with the presence of tissues necrosis caused by disturbance of blood supply or tissue death due to crushing, development of primary or secondary post-traumatic hematomas, which required drainage and led to cover tissues defects, formation of pathological scar tissue on the area of damage and the need for its excision with the impossible use of the primary suture.

Such patients, according to the above-mentioned algorithm of treatment, underwent primary surgical treatment of the wound; after stabilizing of their condition, a full excision of devitalized tissues with a single-stage plastic of the developed defect was performed. In cases of an unstable state of a patient (more than 2-3 weeks), the closure of the cover tissues defect was implemented using the simplest and least traumatic methods of cover recovery.

The method of choosing a plastic material for closure of a wound surface on a lower limb is presented in Table 1.

Cover tissues defects of the distal areas of a lower limb, which were accompanied by a large (more than 1% of the body surface) and significant area of soft tissues traumatization, were the most severe in terms of full and quick damage recovery (Fig. 4).

The highest frequency of cover tissues defects was present in the segment of a leg, 128 (70.3%) patients, and of a foot 36 (19.8%) patients. The hip was traumatized in 18 (9.9%) patients.

This problem was caused by anatomical, hemodynamic and functional features of a lower limb. Firstly, the array of muscles was unevenly situated on the shin, the anterior and

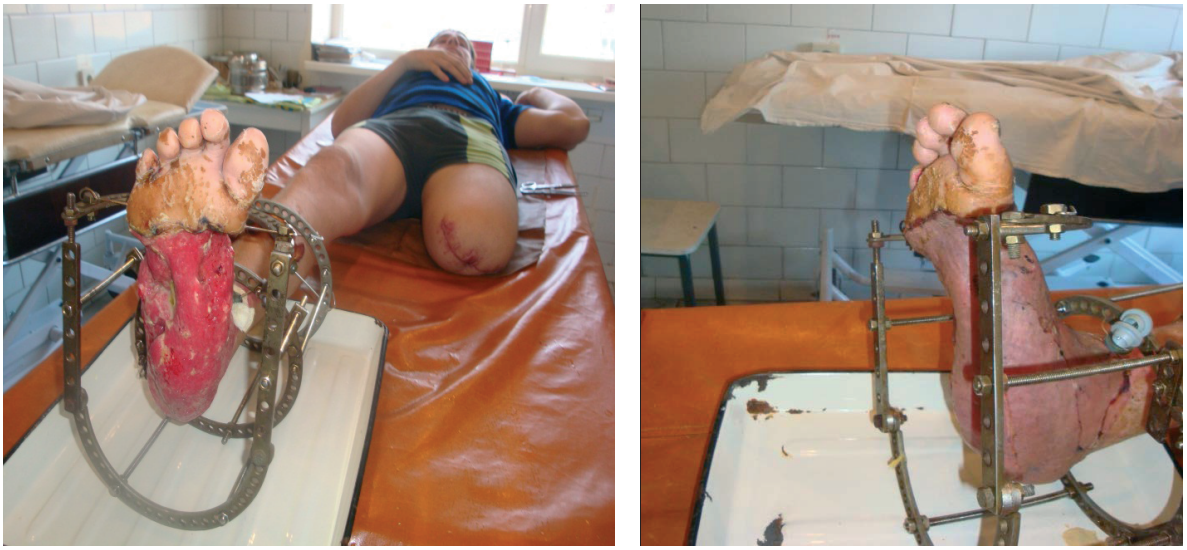

Fig. 4. Patient K., Case No. 19332. Traumatic amputation of the left lower limb at the level of upper third of the leg, crushing of soft tissues, open fragmentary fractures of the bones of the right foot with displacement. Traumatic shock, stage 3. Alcoholic intoxication.

medial surfaces of the tibia were covered only with the skin fascial layer, a lack of soft tissues was present on the foot as well as the specificity of architectonics – a large number of anatomical structures were located in a small space and closely functionally connected with each other.

Secondly, the features of blood supply and the minimum mobility of cover tissues of the distal segments of a lower limb, in cases of combined injuries (83% of patients), did not allow the wound to be sewn by a primary suture, even by moving the skin edges after their mobilization. In that case bone fragments, tendons and muscles were exposed; there was a risk of development of irreversible necrotic purulent inflammatory processes of the osteoarticular apparatus of a limb.

Conclusions

The lower extremity, 182 (182/242; 75.2%) patients, was the most vulnerable segment of the injury. In that case, the defects of cover tissues were characterized by significant anatomical and functional changes, accompanied by bleeding, ruptures and crushing of organs, partial or complete separation of limb segments. The suggested protocol of reconstructive interventions for closure of the defects of cover tissues of lower limbs allowed attaining a positive result in 98.4 % of the interventions.

The prospects for further research go for implementation into clinical practice of full-fledged reconstructive interventions for closure of the defects of traumatic genesis to restore the shape and function of lower extremities in the early stages.

РЕКОНСТРУКТИВНО – ВІДНОВНА ХІРУРГІЯ ВАЖКИХ ПОШКОДЖЕНЬ ПОКРИВНИХ ТКАНИН НИЖНІХ КІНЦІВОК ПІСЛЯ ТРАВМИ

О. В. Пономаренко

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Вступ. *Пошкодження нижніх кінцівок через загрозу інфікування та розвитку гангрени потребують якомога швидшого та ефективного закриття ранової поверхні*

Мета роботи*: розширити покази та покращити результати відновних реконструктивних втручань при важких пошкодженнях покривних тканин нижніх кінцівок механічного ґенезу.*

Методи дослідження. *Протягом 2008-2016 рр. у клініці прооперовано 242 хворих з дефектами покривних тканин тулуба та кінцівок, які виникли в наслідок механічного пошкодження. В залежності від розмірів, глибини та ступеню пошкодження тканин нижніх хворі були розподілені на 4 групи.*

Результати. *Найбільш уразливим сегментом при пошкодженнях були нижні кінцівки – 75,2 % пацієнтів. Тактика Damage Control була застосована у 83 % випадків. При локалізації ран не в проекції функціонально напружених ділянок для закриття дефекту використовували вільну пластику розщепленим шкірним трансплантатом (I група – 12,8 %, II – 20,4 %, III – 37 %, IV – 8,9 %). Якщо рановий дефект зачіпав функціонально значущі структури, такі як оголені кістки та сухожилки, ділянки суглобів та мав площу до 1 % від поверхні тіла використовували складні клапті з місцевих тканин, тканин близьких до дефекту та анатомічно віддалених ділянок (II група – 6 %, III – 10.6 %, IV – 4.3 %). При дефектах понад 1 % від поверхні тіла, складними комплексами тканин закривали лише функціонально напружені ділянки. Решту шкірного покриву відновлювали за допомогою аутодермопластики. На підставі отриманих результатів лікування нами розроблено та запропоновано протокол реконструктивних втручань.*

Висновки. *Впровадження запропонованого протоколу реконструктивних втручань для закриття дефектів покривних тканин нижніх кінцівок дозволило отримати позитивний результат у 98,4 % випадків.*

КЛЮЧОВІ СЛОВА: **травма; дефект покривних тканин; ранова поверхня; клапті; нижні кінцівки.**

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MICROBIOTA OF VAGINA AND MAMMARY GLANDS SKIN IN THE PREGNANT WOMEN WITH PREECLAMPSIA

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Background. *Timely prediction, diagnosis and prevention of complications during the gestational period leading to perinatal loss and maternal mortality are the main tasks of contemporary obstetrics. About 50,000 women die from preeclampsia and eclampsia every year and perinatal mortality ranges from 15 to 25%.*

Objective. *The aim of the research was to study and analyze the microecology of vagina and mammary glands skin in the pregnant women with underlying preeclampsia.*

Methods. *The pregnancy examination was conducted at the Ternopil Regional Clinical Perinatal Center "Mother and Child". The research involved 25 pregnant women with preeclampsia (the main group) and 15 healthy women with a physiological course of pregnancy (the control group). Material from the pregnant women was taken out of the skin of mammary glands and mucous membrane of vagina, then it was plated out in the nutrient medium for the cultivation and the microorganisms were defined.*

Results. *The quantitative composition of a normal microflora of vagina and mammary glands skin in the control group was within the normal range. The quantitative composition of a normal microflora of vagina and mammary glands skin in the pregnant women of the main group decreased, the representatives of opportunistic and pathogenic flora were found.*

Conclusions. *In the pregnant women with preeclampsia, abnormal microbiocenosis of vagina and breast skin was revealed, the degree of abnormality correlated with the severity of the disease. Our results may provide useful clinical knowledge to a broader understanding of microbiota role in pregnancy complications.*

KEY WORDS: **preeclampsia; microbiocenosis of mammary glands skin; perinatal mortality.**

Introduction

In the structure of maternal and perinatal morbidity and mortality, preeclampsia is one of the most leading causes. The incidence of preeclampsia in Ukraine ranges from 6 to 16%. Perinatal mortality in preeclampsia is high and, according to various authors ranges from 15 to 25%. Today, preeclampsia is established to be an inability of the adaptive mechanisms of a parent organism to adequately provide the needs of fetal development, manifested by perfusion-diffuse insufficiency in the mother-placenta-fetus of varying degrees of severity [1, 2, 3, 4, 5].

Preeclampsia is not an independent disease; it is just a manifestation of a systemic inflammatory response syndrome to oxidative stress, endothelial dysfunction, thrombophilia, metabolic homeostasis, and normal microbiota. In the pregnant women with preeclampsia, often a violation of the qualitative and/or quantitative composition of the representatives of normal microflora of the body takes place. This leads to adaptive or irreversible changes in the appropriate microbiological link, which is termed 'dysbiosis' [6, 7, 8, 9].

Physiological colonization is important because normal flora supports immunological homeostasis, suppresses pathogenic microorganisms. The process of formation of microflora of a newborn and the development of its immune system may be disturbed if the expectant mother suffers from pathology of gastrointestinal tract, dysbiosis, has sources of chronic infection, or suffered from preeclampsia during pregnancy. Identifying the beneficial and detrimental microbial components and their roles during pregnancy may have extremely important implications.

According to the literature, there are rare reports on the study of microbiota of mammary gland skin [15, 17], so, the aim of our research was to study the microbiota of vagina and

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mammary glands skin in the pregnant women with preeclampsia.

Methods

The study involved 25 pregnant women with preeclampsia (the main group) and 15 healthy women with a physiological course of pregnancy (the control group). Mild preeclampsia was diagnosed in 15 (60%) of the examined patients, and the severe one was in 10 (40%). Classification of preeclampsia in the pregnant women was conducted in accordance with the Amendment 10 of the ICD (1995), Order of the Ministry of Health of Ukraine dated December 31, 2004 No. 676. Material from the pregnant women was taken out of the skin of mammary glands and the mucous membrane of vagina, then it was plated out in the nutrient medium and the microorganisms were defined.

The examination of the pregnant women was conducted at the Ternopil Regional Municipal Perinatal Center "Mother and Child" in several stages. First, the skin of the mammary glands was rinsed, and the posterior vault of the vagina was smeared from the mucous membrane with sterile swabs pre-moistened in physiological solution. The material was taken by scrolling all the sides of cotton swab. After that, the tampons were placed in sterile tubes and delivered to the laboratory. The time was 20-30 minutes from the time of taking the research material to the crop. The samples were carried out on Petri dishes with sterile media: yolk-salt agar, bloods MPA (for detection of cocci microorganisms), Endo (Enterobacteriaceae), Sabouraud's dextrose agar (fungus of the genus Candida), thioglycollate broth (anaerobic microorganisms). The media was placed in an incubator for 18-48 hours at an optimal temperature. We evaluated the growth of microorganisms in the media after incubation in the incubator (their shape, color, size of the colonies, nature of the surface and edges). Next, smears of certain types of colonies were made, stained with Gram method and examine microscopically.

Results

As a result of microscopic examination, in 15 women of the control group (pregnant women with a physiological pregnancy course) the following was revealed:

1) on the skin of mammary glands: *E. coli, Fusobacterium spp.*, aerobic non–spore-forming gram-positive bacilli, *M. roseus, Streptococcus spp.*, lactose-negative gram negative rods in 6,7% of the examined patients; *Lactobacillus spp., S. haemolyticus* in 13,3%; *S. saprophyticus* in 20,0%; *Corynebacterium spp.* in 26,77%; *Clostridium spp., Bacillus spp.* in 33,0%; *M. luteus* in 40,0%, *Peptostreptococcus spp., S. epidermidis, M. lylae* in 47,0%; *Bacteroides spp.* in 60,0% (Fig. 1).

2) in the vaginal smears: *S. hominis*, Aerobic non spore-forming Gram-Positive Bacillia, *S. haemolyticus* at 6,7%; *Streptobacillus spp.,*

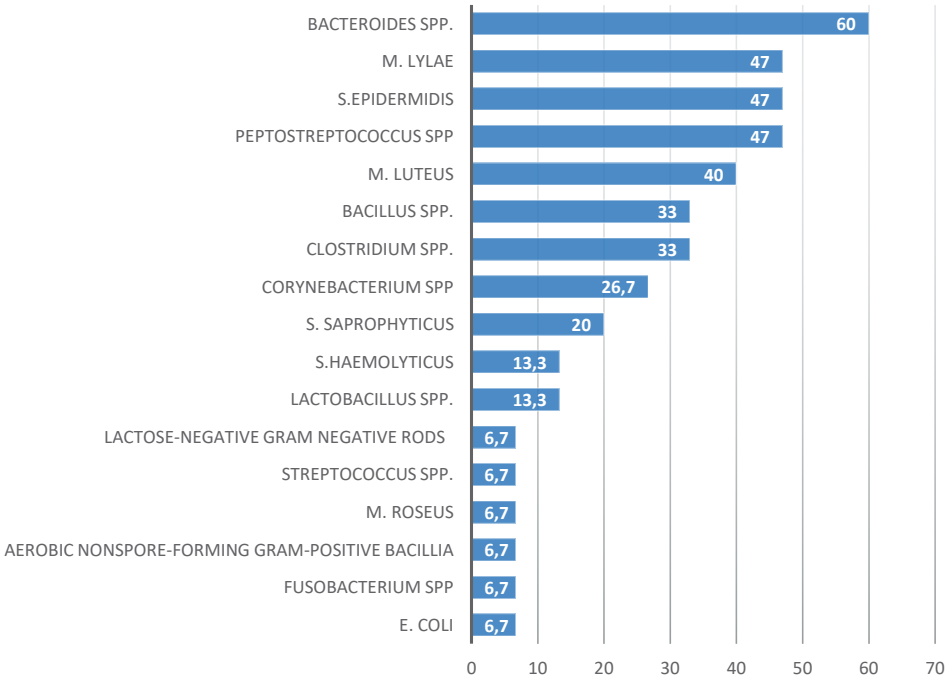

Fig. 1. Colonization of microorganisms on the skin of the mammary glands (control group), %.

Streptococcus spp., M. lylae in 13,3%; *Candida spp., E. coli, S. saprophyticus*, Lactose-negative gram negative rods in 20,0%; *M. luteus* in 33,0%; *Lactobacillus delbrueckii, Corynebacterium spp., S. epidermidis, Bacillus spp*. in 40,0%; *Clostridium spp., Bacteroides spp., Enterococcus spp*. in 47,0%; *Lactobacillus spp*. in 73,3% of the examined women (Fig. 2).

In 15 pregnant women of the main group with mild preeclampsia:

1) on the skin of mammary glands: *Candida spp.* in 6,7%; *M. lylae, β- hemolytic streptococcus* in 13,3%; *Corynebacterium spp., S. saprophyticus* in 20,0%; *S. aureus* in 26,7%; *Bacillus spp., S. epidermidis* in 33,3%; *M. luteus, S. haemolyticus* in 87,0% of the examined women (Fig. 3).

2) in the vagina smears: *S. epidermidis, M. lylae* in 6,7%; *Streptococcus spp., M. luteus*, lactose-negative gram-negative rods in 13,6%, *E. coli, S. aureus* in 26,6%; *S. haemolyticus, Enterococcus spp*. in 33,3%; *Candida spp*. in 46,6%; *Corynebacterium spp.* in 53,3%; *Bacillus spp*. in 60,0% (Fig. 4).

In 10 pregnant women of the main group with severe preeclampsia:

1) on the skin of mammary glands: *Streptococcus spp., Bacillus spp*. in 20,0%; *M. catarrhalis* in 26,6%; *S. epidermidis* in 33,3%; *S. aureus, Corynebacterium spp*. in 40%; *S. haemolyticus* in 66,7%, *M. luteus* in 73,3% (Fig. 5).

2) in the vagina smears: *Corynebacterium spp., P. vulgaris* in 20,0%; lactose-negative gram-

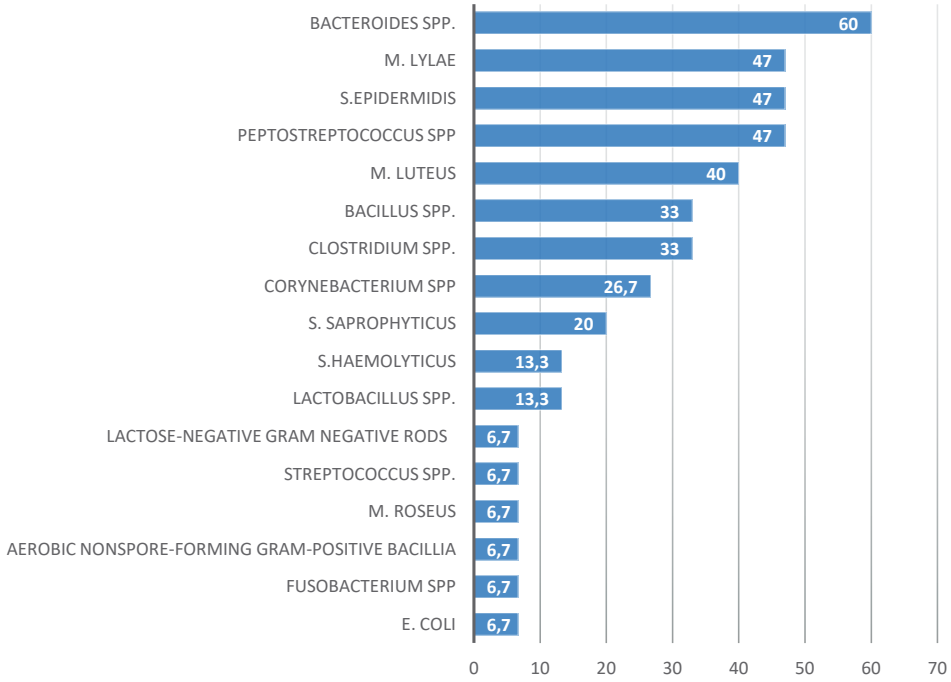

Fig. 2. Colonization by microorganisms of the vaginal mucosa (control group), %.

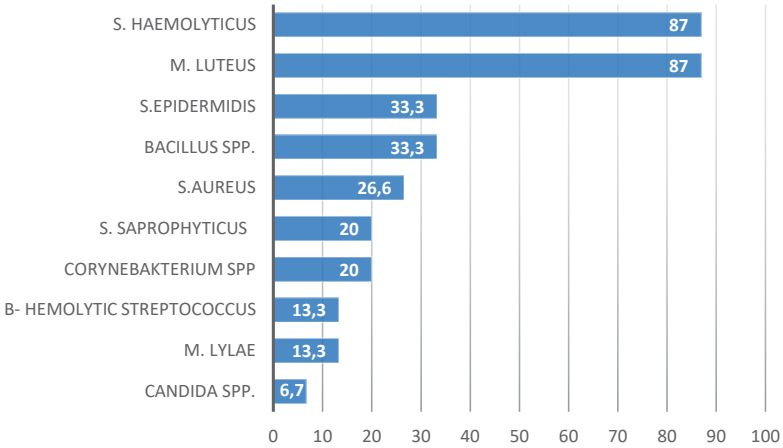

Fig. 3. Colonization of microorganisms on the skin of the mammary glands (the main group, a mild preeclampsia), %.

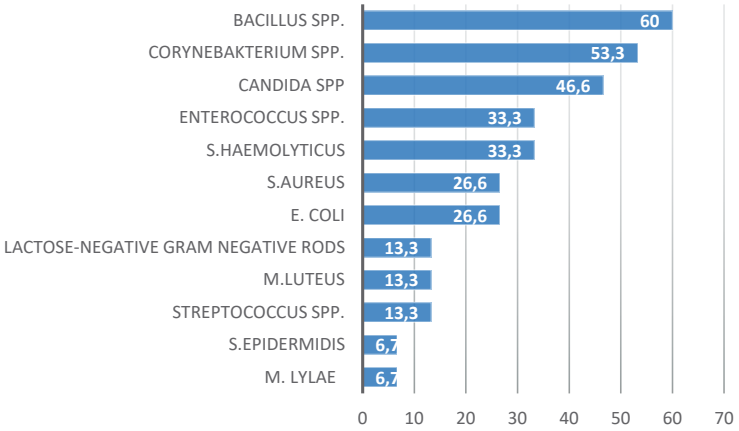

Fig. 4. Colonization by microorganisms of the vaginal mucosa (the main group, a mild preeclampsia), %.

negative rods, *E. coli* in 26,6%; *Candida spp., S. aureus* in 33,3%; *Enterococcus spp., Bacillus spp.* in 46,6%, *S. haemolyticus* in 73,3% (Fig. 6).

Discussion

Since the pathogenesis of preeclampsia is not fully studied, it is practically impossible to prevent its development. However, it is established that in the presence of some risk factors, development of preeclampsia is more frequent. At present, there is no 'gold standard' in the diagnosis of preeclampsia, and maternal and infant mortality from this pathology is still high, therefore, there is a need for further search for new pathogenesis as well as development of screening diagnostic methods.

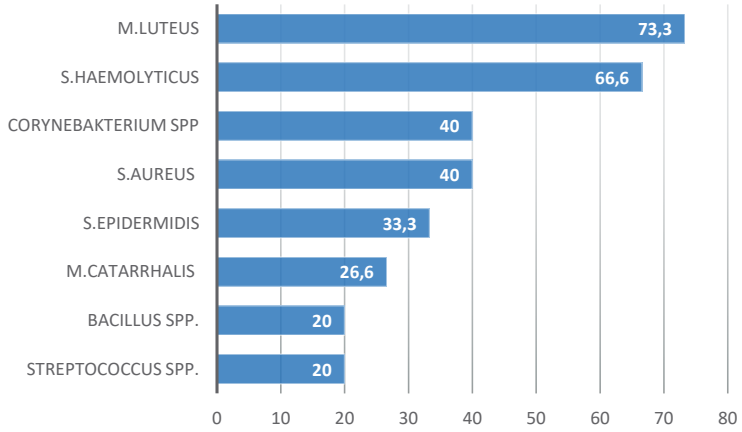

Fig. 5. Colonization of microorganisms on the skin of the mammary glands (the main group, severe preeclampsia), %.

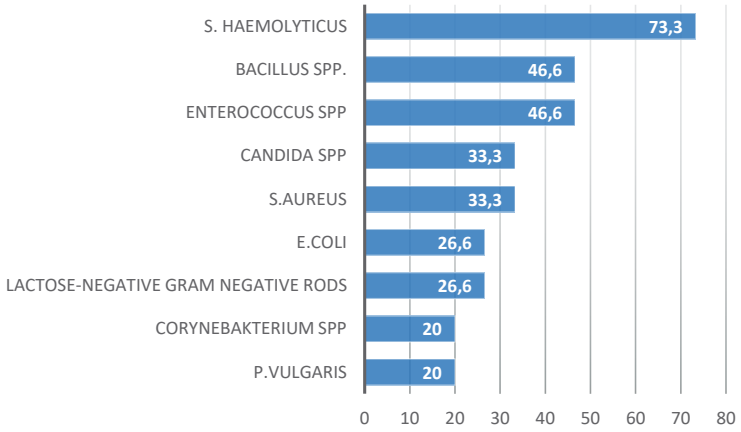

Fig. 6. Colonization by microorganisms of the vaginal mucosa (the main group, severe preeclampsia), %.

The further health care of a woman with preeclampsia is a topical issue. Previously, it was established that the problem of preeclampsia disappears after all. However, according to current literature, this condition has a significant impact on the woman's health and is manifested by the increased risk of development of arterial hypertension, cardiovascular diseases and cerebral circulation disorders in the future [10, 11, 12]. Recent Scandinavian studies have argued that pregnant women, who suffered from preeclampsia before the 37th week of gestation, have an 8-time increase of a risk of cardiovascular death over the next 13 years.

Therefore, it is advisable to recommend to women, who suffered from preeclampsia, an annual examination to evaluate cardiovascular risk as well as keeping a healthy lifestyle in order to avoid long-term consequences of late gestosis [13, 16].

The search for new pathogenesis of preeclampsia would reduce the level of maternal and perinatal mortality and improve the quality of life of pregnant women and childbirth.

Conclusions

The representatives of normal microflora: saprophytic Gram-positive and Gram-negative microorganisms, found on the skin of mammary glands of the control group of women, coincide with the literature data. In the pregnant women with mild preeclampsia, there is an increase in the number of *S. haemolyticus* from 13% to 87%, the presence of the representatives of pathogenic flora: *S. aureus* (in 27% of the examined). In the patients with moderate preeclampsia, the number of *S. aureus* (40%). Depending on the composition of the microflora of a pregnant woman (normocenosis or dysbiosis) and the functional state of mother-placenta-fetus system the microflora of newborns is developed, and their immune system is established.

In the study of microbiocenoses of vaginal mucosa in the examined pregnant women with preeclampsia, in comparison with the control group, a significant decrease in lactic acid bacteria (*Lactobacillus delbrueckii, Lactobacillus spp*.) was evidenced as well as the increase in the incidence of coccal flora (*S. haemolyticus, S. aureus, β-hemolytic streptococcus*). All this evidence the presence of one of the main signs of dysbiosis – a decrease in the frequency of lactic acid bacteria.

Dysbiosis of the skin of mammary glands is found in 87% of the examined of the main group, mucous membrane of vagina – in 73%.

In the pregnant women with preeclampsia, abnormal microbiocenosis of vagina and breast skin was revealed; the degree of changes correlated with the severity of preeclampsia. The analysis of individual variants of microbiota in the examined main group proved that destabilization of a microbial ecosystem takes place before childbirth, which, in our opinion, is associated with changes in the immune system during pregnancy and with certain obstetric pathologies such as preeclampsia. The most unfavorable combination is preeclampsia together with dysbiosis, where significant dysbiotic disorders of both vagina and mammary glands skin with the phenomena of colonization of conditionally pathogenic and transient flora in high diagnostic concentrations are present. It is necessary to make correction of dysbiotic disorders in the pregnant women, and in the future, to maintain normal microflora of the newborns that reduces the number of chronic diseases and slows down the aging process.

МІКРОФЛОРА ПІХВИ ТА ШКІРИ МОЛОЧНИХ ЗАЛОЗ У ВАГІТНИХ ЖІНОК З ПРЕЕКЛАМПСІЄЮ

В. Я. Іванків, І. М. Маланчин, Н. І. Ткачук

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

Вступ. *Своєчасне прогнозування, діагностика та профілактика ускладнень вагітності, які призводять до перинатальних втрат і материнської смертності є важливим завданням сучасного акушерства. Близько 50 тисяч жінок щорічно помирають від прееклампсії та еклампсії, а перинатальна смертність становить від 15 до 25%.*

Мета дослідження *полягала у вивченні та аналізі мікробіоценозу піхви і шкіри молочних залоз у вагітних з прееклампсією.*

Методи дослідження. *У дослідження включено 25 вагітних з прееклампсією (основна група) та 15 здорових жінок з фізіологічним перебігом вагітності (контрольна група) (Тернопільський обласний клінічний перинатальний центр "Мати і дитина"). У обстежуваних жінок брали мазки зі шкіри молочних залоз і слизової оболонки піхви, матеріал висівали в живильні середовища для культивування.*

Результати. *У вагітних основної групи кількісний склад нормальної мікрофлори піхви і шкіри молочних залоз був порушений, виявлено представників опортуністичних бактерій і патогенної флори. Ступінь аномалій мікробіоценозу корелює з тяжкістю клінічних проявів.*

Висновки. *Наші результати дослідження дозволять розширити розуміння ролі і зв'язку змін мікрофлори та ускладнень вагітності.*

КЛЮЧОВІ СЛОВА: **прееклампсія; мікробіоценоз шкіри молочних залоз; перинатальна смертність.**

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OBESITY: A CAUSATIVE RISK FACTOR OF BREAST CANCER

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Background. *Obesity, a global health burden and one of the most deleterious diseases have substantially increased prevalence attributable to changing lifestyle of modern times. Persuasive evidence indicates obesity as an independent prognostic factor for developing malignancy in the form of breast cancer particularly in post-menopausal women.*

Objective. *This review aims to focus in comprehensive manner on the biochemical, hormonal and immunological pathways governing the obesity linked breast cancer so that potential treatments may be improvised consequently to provide a cure to this menace, threatening the lives of many.*

Methods. *Literature review of published materials that provide examination of recent or current literature on problem of obesity.*

Results. *Increased body fatness, mainly visceral adiposity may account for predisposing an obese individual to the risk of encountering cancer although the mechanisms for such cancers may vary depending upon the organ affected. Metabolic and biochemical alterations influencing obesity related carcinogenesis, consisting of heightened oxidative stress and bodily inflammation levels with the concomitant rise in pro-inflammatory cytokines are discussed. Pertinent references about elevated levels of serum insulin, insulin-like growth factor, sex steroids and the imbalance in adipokines (adiponectin and leptin) are included as well.*

Conclusions. *Persuasive evidence indicates obesity as an independent prognostic factor for developing malignancy in the form of breast cancer particularly in post-menopausal women. Generation of novel and effective therapeutic interventions for combating the ailment along with positive lifestyle modifications may be improvised consequently to provide a cure to this menace, threatening the lives of many.*

KEY WORDS: **obesity; breast cancer; lipotoxicity; adiponectins.**

Introduction

Obesity is a major health problem of this century, characterized by excess accumulation of fat due to positive energy balance, resulting from energy intake that exceeds the energy expenditure [1]. A 15-20% of body fat for men and 25% of body fat for women are generally accepted as 'normal', but these are not essentially the optimal values, as a 10% to 20% of excess body fat over the usual values is generally considered to be "obesity" [2].

According to the World Health Organization (WHO) criteria, a BMI greater than or equal to 25 kg/m^2 is overweight, while obesity is defined as having a BMI equal to or higher than 30 kg/m^2. Obesity has been recognized, as a major risk factor for many cancers and, following tobacco use, may be the greatest modifiable cancer risk factor [3, 4, 5]. The incidences of overweight and obesity is dramatically rising in most parts of the world, and is generally higher in women than in men [6]. Convincing data associate being overweight to the risk for various types of cancer as well as other chronic ailments, including cardiovascular disease, stroke and diabetes that are accountable to a large percentage of premature mortality [7, 8]. The International Agency for Research on Cancer reviewed the literature on the involvement between excess body weight and cancer risk. They evaluated the available data as sufficient for a plausible connection with cancers of colon, female breast (postmenopausal), endometrium, kidney (renal cell), and oesophagus (adenocarcinoma). Preliminary information also exists to indicate a relationship with ancillary cancer [9, 10]. Specifically, obesity is related with a twofold increase in the risk of developing breast cancer in case of postmenopausal women while among premenopausal women it is associated with a reduced incidence [11]. Numerous

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interacting hormonal and metabolic pathways seem to underlie the link between being overweight and cancer, with insulin-resistance harbouring a major role. Since evidence is swelling that surplus body weight can also unfavourably influence cancer prognosis, obesity is a prime target for cancer management programs. This review explores the epidemiological and biological evidences concerning the linkage between excess body weight/obesity and particularly cancer in the breast in females, available from several accessible and thorough systematic literature surveys, along with a brief insight into the probable therapeutic interventions in vogue.

Obesity Related Health Disorder

Now a day's obesity and overweight are considered as main causative factors for several chronic diseases, most notably hypertension, type 2 diabetes, dyslipidaemia and coronary heart diseases, osteoarthritis and musculoskeletal disorders, fatty liver, gall stones, psychological disorders and psychosocial problems [12, 13]. Direct relationship of obesity with mortality has also been documented [14]. Among its many health consequences, obesity is increasingly recognized as a risk factor for numerous malignancies, and the obesity-cancer link has recently received much attention [15,16]. Sufficient evidences exist to link obesity with increased risk of colon cancer, postmenopausal breast cancer, endometrial cancer, renal cell cancer and adenocarcinoma of the oesophagus [17].

Obesity and Cancer

World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) concluded that obesity is an established risk factor for several cancers [18]. According to the reports of the last 25 years, obesity was found as a reason of approximately 14% of cancer deaths in men and up to 20% of deaths due to cancer in women [19]. Over this time-period, the commonness of overweight and obesity has gone up from 15% in 1980 to 35% in 2005 [20]. Recent investigations count on the fact that the total health onus of overweight and obesity may surpass that for cigarette smoking [20]. A major review of weight, physical activity, and cancer incidence by the International Agency for Research on Cancer (IARC) concluded in 2002, that obesity was the aetiology of 11% of colon cancer cases, 9% of postmenopausal breast cancer cases, 39% of endometrial cancer incidences, 25% of kidney cancer cases, and 37% of oesophageal cancer incidences [17]. Additionally, data from the American Cancer Society indicated, that overweight and obesity are connected to mortality from liver cancer, pancreatic cancer, non-Hodgkin's lymphoma, and myeloma [19].

Obesity and Cancer – General Mechanism
General Mechanisms of Obesity and Cancer

The cause effect relationship of obesity and cancer are not well known. However, it is well established that it acts through obesity-related hormones, growth factors, multiple signalling pathways of calorie restriction and modulation of energy balance and inflammatory processes. These factors affect the promotion and progression of the cancer cells [21, 22, 23, 24, 25, 26].

Obesity and Breast Cancer

Obesity has been marked as a noteworthy risk factor for breast cancer and the association varies depending upon the menopausal status in females. Breast cancer, as evident from the recent estimates is the most frequent type of cancer in women (28.9% of all female incident cancers) of European population and is the second most common cancer overall [27, 28]. Obesity is found to consistently rise in postmenopausal women by 30%-50% [29, 30, 31, 32]. Breast cancer incidence varies considerably between developed and developing countries which may be attributed to nutritional factors and lifestyle behaviours due to different socioeconomic conditions and variation in ethnicity [33]. Literature clearly indicated the intimate association between obesity and breast cancer that might provide insight in exploring and identifying the various mechanisms involved in this process. Obesity linked breast cancer is multifactorial and involves a network of hormonal and metabolic pathways. Hence understanding the molecular and cellular mechanisms of the obesity-cancer link is imperative for developing potential therapeutics.

Mechanisms Underlying Obesity Related Breast Cancer in Females

Bio-energetic homeostasis and cancer

Metabolic parameters associated with body fatness might influence the bioenergetic balance of the cells and favour the expansion of cells with high anaerobic glycolytic capacity which is a characteristic feature regarding the bioenergetics adaptation of the cancer cells. This effect is termed as "Warburg effect" described by intense lipogenesis and glycolysis and low mitochondrial oxidative phosphorylation capacity even in the presence of adequate oxygen [34, 35]. High blood glucose

levels and hyperinsulinaemia, which is frequent in obese individuals, are thought to pose a selective advantage for the growth of such cells [36]. Increased risk of breast cancer attributed to higher energy intake has been reported in some research studies [37]. Adenosine 5'-monophosphate activated protein kinase (AMPK) is a master sensor of cellular energy status that plays a key role in the regulation of whole-body energy homeostasis [38]. Recently, studies were conducted to examine targets such as AMP activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), fatty acid synthase, deacetylase SIRT1 (sirtuin1) and epigenetic modulators as in nutrient sensing pathways coupled to insulin signalling have been hypothesized to participate in carcinogenesis [19].

Insulin, IGFs, IGFBPs and Insulin resistance – the interplay

Insulin resistance is a metabolic state characterized by a reduced response capacity to insulin by the muscle and liver cells [10]. Insulin resistance has been correlated to a subsequent compensatory excess production of pancreatic insulin leading to hyperinsulinaemia. Chronic hyperinsulinaemia in turn is related to carcinogenesis and linked to breast cancer [39, 40]. This can be explained in the light of the postulates of insulin-IGF hypothesis, which states that chronic hyperinsulinaemia decreases the production of Insulin like growth factor binding proteins (IGFBP1 and IGFBP2) that results in the subsequent rise of plasma levels of free IGF-1 with concomitant alterations in the cellular environment. Both insulin and IGF-1 are anabolic molecules that are capable of tumorogenesis by inhibiting apoptosis, stimulating cell proliferation and motility and being pro-angiogenic [41, 42, 43, 44]. High concentrations of circulating total IGF-1, a major determinant of free IGF-1 concentrations have been associated to an increased risk of premenopausal breast cancer [45]. However, the insulin-IGF hypothesis has two shortcomings. First, levels of total IGF-1 increases linearly with increased BMI but only up to a certain extent around 27 kg/m^2 and thereafter it reduces with further increase in weight [44]. Secondly, in overweight and/or obese individuals, who purposely lose weight (a presumed cancer-protective action), the total IGF-1 concentration tends to escalate the insulin-signalling pathway. This is very much relevant in case of cancer progression because both extracellular signal regulated kinase (ERK) and phosphatidyl inositol-3 kinase (PI3K) pathways are triggered by activation of the insulin receptor (IR). Contrarily, over expression of the IR is evident in breast cancer patients [10, 46, 47, 48, 49]. Insulin and IGF-1 signal by mean of the Akt/PI3K/mTOR cascade for promotion of cell growth and proliferation, thereby inhibiting cell survival [50, 51]. This Akt/PI3K/mTOR cascade has emerged as a target of the obesity and cancer linkage and is activated by both insulin and IGF-1 that are detected frequently at higher concentrations in the serum of the overweight and obese individuals, culminating [52, 53, 54].

Alterations in sex hormones

Steroid hormones including oestrogen, progesterone, androgens and adrenal steroids are related with energy homeostasis and obesity related progression of different types of male and female cancers [55]. Obesity increases the risk of developing breast cancer after menopause and it has been indicated that up to 50% of postmenopausal breast cancers are linked to obesity [37]. Predisposing risk factors familiar in developing breast cancers are related to oestrogen e.g., early menarche, late menopause and hormone replacement therapy (HRT) [56, 57, 58, 59]. Obesity and age has been ascertained as factors that may negatively influence the survival of patients with breast cancer [60,61]. Increased adiposity may influence sterol synthesis and metabolism of oestrogens. Obesity has been associated with increasing levels of oestrogen because of accelerated peripheral aromatization of adrenal androgens in adipose tissue among postmenopausal women, that can promote cell proliferation, have anti-apoptotic and pro-angiogenic effects [62,63]. In postmenopausal women, plasma levels of free oestradiol and testosterone are positively associated to breast cancer occurrence [64]. Studies revealed that the relationship between obesity and breast cancer risk in postmenopausal women might be justified by heightened levels of oestrogens, particularly bioavailable oestradiol [65, 66]. Further, in case of postmenopausal women the link between body mass index (BMI) and risk of breast cancer has been strongly evident among women, who do not use hormone replacement therapy (HRT), compared to women, who have undergone HRT [67]. Some studies showed an inverse relationship between BMI and pre menopausal breast cancer and this may be supported by the fact that for pre menopausal women obesity is linked with a higher frequency of anovulatory cycles and with reduced levels

of circulating sex steroids [67]. Another dimension to the association between BMI and breast cancer is mammographic density, the latter being negatively correlated with BMI. For adjustment of mammographic density, estimates for BMI, cancer risk rise [68].

Lipotoxicity

Cancer cells exhibit accentuated de novo lipogenesis by means of elevated fatty acid synthase (FASN), an enzyme responsible for synthesizing endogenous fatty acids, that may be modified and packaged into structural lipids required for cell division [69]. Both obesity and cancer cell-derived lipolytic enzymes produce free fatty acids for the tumour to supply structural as well as oncogenic lipid signalling molecules such as platelet activating factor (PAF), sphingosine 1-phosphate (S1P), lysophosphatidic acid (LPA) and prostaglandins [70]. Elevated FASN enzyme, mRNA, and enzymatic activity have been documented in human breast cancer cell lines and the rise in FASN is thought to be essential for evoking the malignant effects of proliferation and survival although this alone is not the reason for malignancy [71]. Thus elevated basal lipolysis followed by increased plasma levels of free fatty acids (FFAs) leads to enhanced intracellular accumulation which can impair non-adipose cells in their normal role as well as insulin signalling and the phenomenon is known as "lipotoxicity" [72].

Obesity induced immunosuppression

Obesity induces chronic, low-grade inflammation leading to increased levels of local and systemic proinflammatory cytokines including prostaglandin E2 (PG E2), tumour necrosis factor-alpha (TNF-α), interleukin (IL-2, IL-8, IL-10), C-reactive protein (CRP) and monocyte chemoattractant protein (MCP-1). In this context activation of NF-κB complex may be cited as a possible mechanism by which inflammation may stimulate cancer progression [24,25]. Thus, the proinflammatory state evident in the metabolic cells of adipocyte and the recruitment of immune cells along with the consequent release of inflammatory cytokines (TNF-α, IL-6, adiponectin etc.) is the outcome of obesity.

Tumour necrosis factor–α or TNF–α

A pro-inflammatory cytokine by nature TNF-α exerts several effects in adipose tissue encompassing lipid metabolism and insulin signalling in which the circulating levels are elevated with obesity and levels off with weight loss. A rise in TNF–α stimulates the secretion of other pro-inflammatory cytokines like IL-6 while decreasing the levels of anti-inflammatory cytokines like adiponectin [73]. Research findings indicated that TNF-α promoted adipocytes apoptosis and induced insulin resistance by means of inhibiting the insulin receptor substrate 1 signalling pathway [74,75].

Interleukin–6 or IL–6

Macrophage is the preliminary source of circulating IL-6 that plays a pivotal role in the whole-body energy homeostasis, as well as inflammation. The fact that IL-6 has the potential to suppress the activity of lipoprotein lipase has been deduced from both in vitro and in vivo studies. Expression of IL-6 receptor is evident in certain brain regions and hypothalamus being one of them is responsible for controlling appetite and energy intake [76].

Adiponectin

Contrary to the reduced levels of adiponectin as seen in cases of animal models of obesity and insulin resistance, weight loss has been found to elevate the adiponectin levels. Regulation of lipid and glucose metabolism, increased sensitivity towards insulin, body weight and food intake regulation and protection against chronic inflammation are some of the vital roles of adiponectin [77].

Intracellular pathways of inflammation

Overfeeding has been hypothesized to be the starting signal of inflammation in obesity and the pathway has its inception in the metabolic cells like the adipocyte, hepatocyte or myocyte. Acute evocation of inflammatory responses due to consumption of nutrients has been suggested from studies in mice and humans [78, 79]. Adipose tissue and liver in obese men and women, when compared to lean controls, exhibit hyperactivation of three kinases, namely: the c-jun N-terminal kinase (JNK), the inhibitor of K kinase (IKK) and the protein kinase R (PKR) capable of inducing inflammatory cytokines' expression [80, 81]. The inflammasome and the Toll-like receptors (TLRs) of the innate immune system are activated as well in those same metabolic tissues [82, 83, 84]. Inflammatory signals or nutrients may trigger off the TLRs pathways and downstream JNK, IKK and PKR. These kinases control downstream transcriptional programs by means of the transcription factors activator protein-1 (AP-1), NF-κB and interferon regulatory factor (IRF) inducing upregulation of inflammatory mediator gene expression. The rise in cytokines aggravates receptor activation through a positive feedback loop of inflammation and the inhibitory signalling of metabolic pathways [85].

Dysregulation in adipokines

The adipose tissue, known primarily as energy storage organ, by virtue of recent studies has also been established as an endocrine organ, producing and secreting polypeptide hormones, adipokines, among which leptin and adiponectin are most common and involved in cancer development [86]. Adipokines (leptin, adiponectin and hepatocyte growth factor (HGF) are recognized for their participation in the mechanisms by which obesity and related metabolic disorders affect breast cancer risk [87]. The physiological and pathological communications of leptin and adiponectin are mostly antagonistic, as are their biological consequences on breast cancer cells [88].

Leptin, a hormone essentially exclusive to adipose tissue acts centrally in the hypothalamus for regulation of body weight and peripheral energy expenditure [87, 89]. Circulating leptin levels are strongly correlated to the body fat content and are prominent in obese subjects to normal individuals [90, 91, 92]. Thus leptin, a potential mediator of obesity-related cancer influences cancer progression by activating PI3K, MAPK and STAT3 pathways, while the stimulatory effects of leptin on breast cancer growth were noted to occur primarily via oestrogen receptor activation [21, 26, 88, 93, 94]. Further, evidences through extensive research suggest that adiponectin, the most abundant adipokine, affect the proliferation and insulin sensitivity of various types of cells [95]. Unlike leptin, adiponectin is inversely related with adiposity, hyperinsulinaemia and inflammation [22]. Moreover, adiponectin may incur anticancer effects by diminishing insulin/insulin like growth factor (IGF-1) and mTOR signalling via activation of 5' AMP-activated protein kinase (AMPK) and providing anti-inflammatory action by the inhibition of nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) [22]. Current findings indicate that the low serum adiponectin levels are significantly associated with an increased risk for breast cancer and that tumours arising in women with the low serum adiponectin levels have greater likelihood of expressing a biologically aggressive phenotype [95]. Another adipokine, hepatocyte growth factor (HGF) or 'scatter factor' may exert a positive influence on tumorigenesis as a consequence of its anti-angiogenic properties but is mainly known for its ability to promote cell invasion [88]. Numerous investigations revealed that the serum concentration of HGF are often elevated in patients with breast cancer and particularly so in those suffering from the advanced disease stage [96, 97, 98].

Conclusions

The striking association between obesity and incidence of breast cancer has been established through several investigations and experiments until date. The various metabolic and endocrine mechanisms that account for the pathogenesis of obesity linked breast cancer have been discussed here to further probe into the nodal points of control in these cascades that may be beneficial to the researchers for generation of novel and effective therapeutic interventions for combating the ailment along with positive lifestyle modifications. Currently hormonal therapy with selective estrogen receptor modulators (SERMs) (such as tamoxifen and raloxifene) as well as aromatase inhibitors (such as exemastane, anastrozole, and letrozole) has been approved as standard mode of treatment of women with estrogen receptor-positive breast cancer. This therapy alongside adjuvant therapy acts in curing of advanced disease form though issues relating to their side effects are also a major concern [99, 100]. The efficacy of another drug which acts as an insulin lowering agent named metformin, in reducing breast cancer recurrence is presently being studied extensively [101, 102, 103]. Simultaneously in the recent years Yoga based lifestyle interventions that is a form of physical activity facilitating in accomplishing recommended levels of physical fitness have gained much attention and are found to effectively thwart and hinder the progression of cardiovascular and metabolic syndromes like that of obesity [104, 105]. The method of action of such benefit may be credited to a reduction in weight and stress, networking at mind and body levels, thereby leading to a decline in inflammation, and causation and progression of the disease [106]. Thus, any further information about the drugs and other treatment modalities that can ameliorate the adverse effects of breast cancer by altering the markers of obesity may also be useful in this regard.

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ОЖИРІННЯ – ФАКТОР РИЗИКУ РАКУ МОЛОЧНОЇ ЗАЛОЗИ

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Вступ. *Ожиріння – це глобальна проблема здоров'я, що набула значної поширеності зі зміною сучасного способу життя. Також це незалежний прогностичний фактор розвитку раку молочної залози, особливо у жінок в період постменопаузи.*

Мета *огляду – комплексний аналіз біохімічних, гормональних та імунологічних чинників, які пов'язують ожиріння з раком молочної залози, та пошук потенційних методів лікування.*

Методи дослідження. *Аналіз даних літератури для оцінки поточного стану проблеми.*

Результати. *Надмірна вага, та головним чином накопичення вісцерального жиру, пов'язані з підвищеним ризиком розвитку злоякісних захворювань, однак механізми їх розвитку значно варіюють залежно від ураженого органу. Обговорюються метаболічні та біохімічні показники, що впливають на канцерогенез, пов'язаний з ожирінням; включно з розвитком оксидативного стресу та ознак запального процесу з одночасним підвищенням рівня прозапальних цитокінів. А також такі фактори як підвищений рівень сироваткового інсуліну, інсуліноподібного фактора росту, статевих стероїдів та дисбалансу адипокінів (адипонектину і лептину).*

Висновки. *Переконливі докази вказують на ожиріння як незалежний прогностичний фактор розвитку раку молочних залоз, особливо у жінок після менопаузи. Розробка нових ефективних терапевтичних заходів для лікування раку та модифікація стилю життя можуть забезпечити позитивні зрушення.*

КЛЮЧОВІ СЛОВА: **ожиріння; рак молочної залози; ліпотоксичність; адипонектини.**

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DYNAMICS OF PERIODONTAL TISSUES MICROBIOCENOSIS UNDER THE COMPLEX TREATMENT OF CATARRHAL GINGIVITIS AND CHRONIC GASTRODUODENITIS IN THE ADOLESCENTS

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Background. *The key links in the etiology and pathogenesis of periodontal tissue diseases are the quantitative and qualitative changes in the composition of the microflora of oral cavity, with the simultaneous deterioration of oral hygiene, reduction of local and general immunity, which occurs more often in the presence of somatic diseases.*

Objective. *The aim of the research was to determine the clinical and microbiological efficacy of the developed treatment-prophylactic complex in the adolescents with catarrhal gingivitis and chronic gastroduodenitis before and after treatment.*

Methods. *Changes were made to and before the treatment of clinical parameters, gingival microbiocenosis of 38 adolescents with generalized catarrhal gingivitis and chronic gastroduodenitis aged 12-18 years old, who comprised the main group. In the comparison group 25 adolescents of similar age diagnosed with generalized catarrhal gingivitis without any somatic diseases were involved.*

Results. *It has been established that used combination (drug of plant origin with antimicrobial properties + dental gel with Metronidazole benzoate and Chlorhexidine digluconate + capsules of probiotics) yields the conventional treatment as well as exceeds it for examined clinical indicators and indexes. The treatment and prophylaxis with suggested complex have proved a significant positive effect on the gums microbiocenosis in adolescents with generalized catarrhal gingivitis and underlying concomitant gastroduodenitis.*

Conclusions. *The suggested therapeutic and prophylactic complex provides a reduction in the massiveness and colonization frequency of the gum mucosa by pathogenic aerobic microflora (β-hemolytic streptococcus, golden staphylococcus, and yeast-like Candida fungi).*

KEY WORDS: **catarrhal gingivitis; chronic gastroduodenitis; adolescents; microbiocenosis; complex treatment.**

Introduction

Recent epidemiological studies prove a high intensity and prevalence of periodontal tissue diseases in childhood. According to many authors, children are mainly diagnosed with chronic catarrhal gingivitis, which prevalence reaches 90% [6, 13, 19, 20]. According to the contemporary concept, the development of periodontal tissue diseases is closely related to the microflora of the oral cavity, that is the reduction of the number of normal flora, the increase of conditionally pathogenic microorganisms, excessive indigestion and infection with periodontopathogens with simultaneous deterioration of oral hygiene, reduction of local and general immunity in the presence of somatic diseases are key links of etiology and pathogenesis of the disease [1, 4, 11, 12, 16]. At the present time, the normophyll of the human body is considered to be a combination of microbiocenosis, which is part of a holistic system that performs the most important functions in the body: it is a supplier of biologically active substances, a powerful metabolic and detoxification body, it determines the formation of the overall immunological status of the organism and local immunity, and most importantly creates an anterior line of nonspecific microorganism protection [3, 17, 22, 23]. The oral microbiome is one of the most complex and diverse microbial communities in the human body. In the development of dysbiosis disorders of the organism, conditionally pathogenic microbes prevail, among which are clones with medicinal resistance and genetic determinants that determine the virulence and pathogenicity of bacteria, but it is established that streptococci (representatives of normal flora) in the stage of primary inflammation of

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the gum are significant in the development of a pathological process, for example, the fixation of *P. gingivalis* and *P. intermedia* on the gum surface occurs after the appearance on these areas of *Streptococcus mitis* and *Streptococcus sanguis*, which contribute to attachment of parodontal microflora forming an intermediate layer between them and the outer membrane of epithelial cells [8, 14, 18].

The issue of creation of effective integrated treatment schemes is urgent, due to defeats in treatment, lack of a stable clinical effect, presence of relapses, resulting in a one-way approach to treatment without considering the features of the existing microflora, characteristics of local resistance and general condition of the organism [2, 10, 21].

The aim of the research was to determine the clinical and microbiological efficacy of the treatment and prophylactic complex aimed at correction of microbiocenosis of periodontal tissues in the adolescents with catarrhal gingivitis and chronic gastroduodenitis by clinical and microbiological monitoring before and after treatment.

Methods

A clinical dental examination of 38 adolescents with generalized catarrhal gingivitis and chronic gastroduodenitis aged 12-18 years old, who comprised the main group, was conducted (group 1). In the comparison group, 25 adolescents of the same age diagnosed with generalized catarrhal gingivitis, who, at the time of the survey, had no complaints of violations of somatic health and were not on the dispensary records of related specialists, were involved (group2). As a control, similar studies were performed in 20 adolescents of the same age without any signs of gum inflammation and somatic diseases.

Clinical examination of the adolescents was carried out according to the generally accepted technique using subjective and objective methods. The received data of each patient was entered in an outpatient documents of a dental patient and the record of our examination developed by us. At an objective dental examination of the patients, the depth of the pride of oral cavity and the features of the attachment of bridles, bite, tooth row and its integrity, the presence of seals and their condition were studied. Particular attention was paid to the condition of the gums: color (pale pink, hyperemia, cyanosis), relief of the gingival margin (exacerbation of the peaks of gingival papillae, conical incision, swollen papillae) and consistency (normal tone, edema, pastiness), a kind of gingivitis, prevalence. The evaluation of the condition of gums around the tooth was carried out by sounding a periodontal probe. The index score was used to determine the initial state of periodontal tissues in the pre-existing groups and during monitoring after treatment. In order to assess the oral hygiene status, all patients were given the Simplified Oral Hygiene Index (Green-Vermillion, 1964), which allowed them to detect not an only plaque but also a tooth_brush. To evaluate the inflammatory process in gums, the PMA index (papillary-marginal-alveolar index by C. Parma, 1960) was used. To establish the diagnosis and prognosis of the treatment of periodontal tissue diseases Papillary Bleeding Index (Saxer and Muhlemann, 1975) was used.

Simultaneously, microbiological studies were carried out on the contents of the tooth-asparagus furrow. A material for bacteriological examination for revealing of aerobic and extra-anaerobic microflora from the tooth-asparagus was conducted on tooth brushing, using a calibrated bacteriological loop No.1 on blood agar, Endo medium, and a potassium-iodine-starch indicative medium system (for identification of producers of hydrogen peroxide) and it was delivered to a microbiological laboratory within an hour. Plating was performed by the Gold method [9].

The seeds were incubated for 1 day at 37 °C under aerobic and anaerobic conditions (in a hermetically sealed desiccator) in an atmosphere enriched with CO_2. The bacteriological examination was carried out in order to isolate pure cultures of microorganisms and their identification according to generally accepted microbiological methods for bacteria identification [5]. Identification of the isolated pure cultures was carried out by a complex of morphological, cultural and biochemical methods (STREPTOtest 16, STAPHYtest 16, Erba Lachema, Czech Republic). Quantitative records of colonies were conducted according to their species (or generic) affiliation. The results of the quantitative study of microflora were registered in colony-forming units, converted to 1.0 ml – CFU/ml, taking into account only those microorganisms which concentration in the specimen was not less than 1×10^3 CFU/ml. Based on the analysis of crop yields for each group, the population level PL (lg CFU/ml) and the Continuous Index (CI) [5] were determined.

Integrated therapy of GCG was carried out in accordance with the protocols approved by

the Order of the Ministry of Health of Ukraine No.566 dated 23.11.2004 "On Approval of Protocols for the Provision of Medical Aid to Children for the Specialty Pediatric Therapeutic Dentistry". The patients of the main group and the comparison group were divided into A and B subgroups according to treatment schemes. Patients of 1A and 2A subgroups were prescribed a combined herbal antimicrobial medicine in the form of paddling with 15% aqueous solution (about 10 ml of the preparation dissolved in ¼ cups of water) of the oral cavity 3-4 times a day, application to the gum mucosa and introduction into the interdental dentately gaps 2 times a day. Combined herbal antimicrobial medicine is a mixture of a mixture of chamomile flowers, oak bark, sage leaves, Arnica herbs, Ayer rhizomes, peppermint herbs, and Thyme grass. For general treatment, probiotics (capsules of Yogurt) were prescribed 1-2 capsules 3 times a day with a meal. For local treatment of the patients of 1B and 2B subgroups, irrigation of the gums was used with 0.05% chlorhexidine digluconate solution, herbs (chamomile, calendula) 3-4 times a day for 7 days, applications on the gum mucosa and insertion into the interdental gaps' ointment with mefenamic acid 2 times a day. The course lasted 10 days.

The data were expressed as the mean±standard error of the mean (M±m). Probability values with $p<0.05$ were considered statistically significant. The distribution of indices was estimated by using the Shapiro-Wilk Normality Test. The statistical significance of the differences between means was assessed by Student's T-criterion using Statistica 5.0 (Statsoft, USA).

The research was carried out in accordance with the principles of the Helsinki Declaration. The protocol of the study was approved by the Local Ethics Committee (LEC) of all institutions mentioned in the work. In accordance with the requirements of bioethics "On conducting laboratory research of biological material", written consent was received from the parents (guardians) of each child and the adolescents for the study of biomaterials.

Results

According to the results of the clinical examination, the prevalence of catarrhal gingivitis in the adolescents of the main group was higher than in the comparison group, 69.8% versus 52.7%, respectively. The course of gingivitis in the patients of the main group in most cases was chronic or in the stage of aggravation, of moderate severity, with the main complaint of bleeding gums. In the comparison group, mild chronic catarrhal gingivitis was predominantly diagnosed.

The PMA index evidenced that the degree of severity of gingivitis was higher in the adolescents of the main group – 36.8±1.21%, which corresponds to the average severity of gingivitis, 19.2±1.07% in the adolescents of the comparison group, which corresponds to mild gingivitis. The mean value of bleeding index was 1.23±0.01 points in the main group and 0.8±0.01 points in the comparison group.

The correlation between the level of oral hygiene and the prevalence of inflammatory events in periodontal tissues was defined. Analyzing the results of oral hygiene state, it was found that the average value of hygiene index in the adolescents of the main group and the comparison group was a satisfactory and unsatisfactory condition of the oral cavity. Thus, in the adolescents of the main group, the average index was 1.76±0.03 points, in the comparison group – 1.32±0.03 points. After the treatment, oral hygiene improved by 0.31±0.04 points and 0.17±0.02 points in the main and in the comparison group respectively, which corresponded to a good oral hygiene condition.

At the end of the course of complex treatment and elimination of clinical manifestations of the disease, the complaints in all adolescents were absent. Gums were pale pink, of a dense-elastic consistency, did not bleed when probing in the area of the tooth-spatula furrow. However, the adolescents of subgroups 1A and 2A, who received the suggested improved treatment, were more likely to have shortened treatment terms than the adolescent of subgroups 1B and 2B receiving traditional treatment.

During the treatment, all patients of the main group, as well as the comparison group, proved a positive dynamic of the studied parameters. Thus, the dynamics of the PMA index tended to reduce the signs of inflammation: the value of the PMA index after the end of treatment in the adolescents of the main group, subgroup 1A was 3.7±0.12% and the subgroup 1B – 6.8±0.14%. In the adolescents of the comparison group, subgroup 2A it was 1.6±0.08% and the subgroup 2B – 2.9±0.13%. A similar trend was evidenced in the study of the dynamics of the index of bleeding: the index after the end of the treatment course in the adolescents of the main group, subgroup 1A was 0.11±0.02 points and the subgroup 1B –

0.17±0.03 points. In the adolescents of the comparison group, subgroup 2A it was 0.07±0.01 points and the subgroup 2B – 0.17±0.02 points.

However, in 6 months of monitoring, a slight worsening in clinical performance was evidenced in all groups, but in the adolescents of groups 1A and 2A, the increase in clinical indices was less significant than in the groups 1B and 2B. Thus, the PMA index for the adolescents of groups 1A and 2A in 6 months was 4.5±0.11% and 2.4±0.12% respectively, which was less than in the adolescents of groups 1B and 2B – 7.4±0.25% and 4.5±0.14% ($p<0.01$), respectively. The abnormal pattern was evidenced in the study of the iodine of gums bleeding: in the adolescents of groups 1A and 2A in 6 months it was 0.23±0.01 points and 0.18±0.01 points, respectively, which was less than in the adolescents of groups 1B and 2B – 0.31±0.01 points and 0.27±0.01 points ($p<0.01$), respectively.

The initial microbiological examination proved that, compared to the control group, in the adolescents with generalized catarrhal gingivitis, both in the context of gastroduodenitis and without concomitant pathology, a higher level of colonization of gum mucus by the representatives of resident α-hemolytic streptococci ($p<0.01$) and transient microflora of the oral cavity: epidermal staphylococcus ($p<0.05$), stomatococcus ($p<0.05$), and *Corynebacterium* (diphtheroids) ($p<0.05$), was evidenced. In addition, the presence of active inflammatory process on the gum mucosa is accompanied by a significantly higher level of colonization of the affected areas by *Staphylococcus aureus* ($p<0.05$), β-hemolytic streptococci ($p<0.05$), and yeast-like fungi of the genus Candida. In this regard, a therapeutic and prophylactic complex aimed at correction of microbiocenosis of periodontal tissues (patients of subgroups 1A and 2A) was developed. The effectiveness of the suggested complex was evaluated by a comparison of the dynamics of microbiological parameters with patients of subgroups 1B and 2B, who received protocol traditional treatment. By quantitative indicators of microbiocenosis of gum mucosa (population level and index of the constancy of different microorganisms), obtained during the initial examination before treatment, the comparable subgroups of patients (1A and 2A, 1B and 2B) practically did not differ.

The main representatives of the resident microflora of the oral cavity: α-hemolytic streptococci were plated in all patients without exception in all periods of the monitoring. However, both therapeutic complexes demonstrated significant reducing the massiveness of colonization of gum mucus by α-hemolytic streptococci (Fig. 1).

The most significant dynamics was evidenced after the treatment in the patients of subgroups 1A and 1B: population-level decreased by 6.43±0.15 lg CFU/ml to 4.72±0.13 lg CFU/ml ($p<0.01$) and from 6.27±0.15 lg CFU/ml to 5.7±0.15 lg CFU/ml ($p<0.01$), which corresponds to normal age indices in the control group without dental pathology. Significant positive dynamics was also evidenced in the patients of subgroup 2B (population level decreased from 5.05±0.26 lg CFU/ml to 4.23±0.13 lg CFU/ml, $p<0.05$). However, in the latter case, PL α-hemolytic streptococci dipped below the age standard (4.74±0.31 lg CFU/ml).

During a long-term after treatment period (in 6 months), the level of colonization of mucosal α-hemolytic streptococci in subgroups 1B and 2A increased again ($p<0.05$). The patients of the subgroup 1B did not experience any

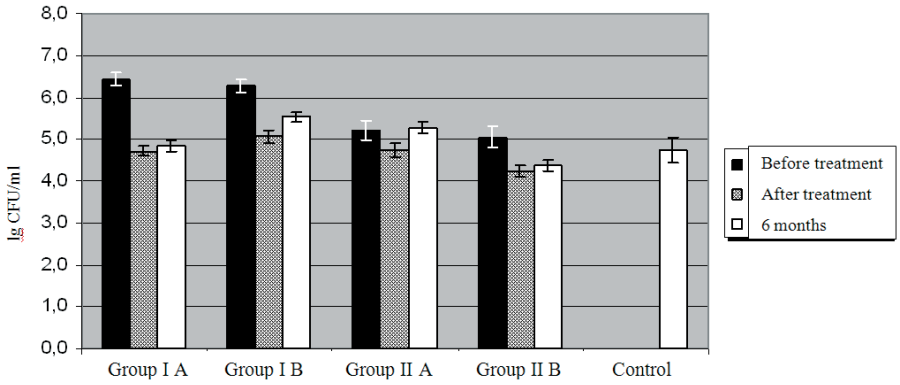

Fig. 1. Dynamics of population-level changes (PL) of α-hemolytic streptococci on gingival mucosa of the adolescents with GKG in the use of various therapeutic complexes.

improvement; however, the α-hemolytic streptococcal fraction was below the age standard. In the patients of the subgroup 1A (patients with ketal gingivitis and underlying gastroduodenitis), the suggested therapeutic complex allowed achieving a stable normalization of this index (PL α-hemolytic streptococci 4.84±0.12 lg CFU/ml, p<0.01 compared to the pre-treatment stage).

It should be noted that the suggested therapeutic complex also contributed to normalization of the species composition of α-hemolytic streptococci on gingival mucosa of the adolescents followed up. Thus, before treatment of catarrhal gingivitis 68.4±3.32% of the patients of the main and 64.0±3.43% of the comparison group, *Streptococcus gordonii, Streptococcus sanguis, Streptococcus constellatus, Streptococcus anginosus* were isolated (which, in comparison with other α-hemolytic streptococci, have wider sets of factors of virulence). The overwhelming majority (96.4±2.3%) of α-hemolytic streptococci cultures from dermatologically healthy individuals were defined as Streptococcus salivarius and Streptococcus mitis. After the treatment, in the patients with scar tissue of subgroups 1A and 2A, the latter increased to 84.6±3.2% and 76.5±3.9% respectively.

In the patients treated for GCG in the traditional way (subgroups of 1B and 2B), there was a significant decrease in the massiveness of colonization of gum mucosa by transient representatives of normal microflora of oral cavity: stomatococcus, non-series, and diphtheroids (Table 1). In the long term after treatment period (in 6 months), low population levels of these representatives of normophyll in the patients of the subgroups 1B and 2B were still present. At the same time, they were lower than the age norm, which may evidence a stable deficit of minor representatives of normal microbiocenosis of the oral cavity. The dynamics of changes in the index of constancy (plating frequency) of these microorganisms in the subgroup 2B was similar (Table 2).

On the contrary, the massive colonization of gum mucus by epidermal staphylococci in the treatment of the patients of subgroups 1B and 1B, on the contrary, proved a tendency to increase (Table 1). Nevertheless, the used therapeutic measures allowed achieving a short-term decrease in the frequency of plating *S. epidermidis* from the gum in these groups (Table 2).

The suggested new therapeutic complex, which included milder local action of antiseptic agents in combination with probiotics, proved a gentler normalizing effect on germ microbiocenosis. In the patients of subgroup 1A (GCG with underlying gastroduodenitis) immediately after the course of treatment and in 6 months, the rate of PR and IE representatives of transient microflora of oral cavity was close to normal age values (Tables 1 and 2).

Table 1. The massiveness of gingival mucosa colonization with transient representatives of the oral cavity normal microflora (population level, lg CFU/ml) during treatment of the adolescents with GCG

Patients subgroups		*S. epidermidis*	*Stomatococcus mucilaginosus*	*Neisseria sp.*	*Corynebacterium sp.*
Control		3.78±0.21	3.39±0.08	3.57±0.22	3.00±0.05
GCG+Gastroduodenitis					
1 A	Before treatment	4.89±0.24 †	4.83±0.38 †	3.93±0.16 †	4.20±0.16 †
	After treatment	3.68±0.09 *	3.57±0.14 *	3.57±0.12 *	3.00±0.05 *
	In 6 months	3.89±0.24 *	3.68±0.15 *	3.50±0.13 *	3.47±0.09 */†
1 B	Before treatment	4.18±0.30	4.78±0.30 †	3.74±0.27	4.00±0.19 †
	After treatment	4.05±0.24	3.45±0.12 *	4.35±0.11 */†	3.00±0.05 *
	In 6 months	4.38±0.17 †	3.69±0.07 *	3.14±0.07 */†	3.50±0.16 */†
GCG					
2 A	Before treatment	3.43±0.15	4.03±0.13 †	4.13±0.27 †	3.85±0.06 †
	After treatment	3.43±0.24	3.73±0.11 */†	3.35±0.11 *	3.57±0.14 †
	In 6 months	3.60±0.17	3.83±0.04 †	3.35±0.14 *	3.60±0.12 †
2 B	Before treatment	3.94±0.27	4.76±0.27 †	3.85±0.35	3.80±0.25 †
	After treatment	3.90±0.25	3.57±0.15 *	3.00±0.05 */†	3.00±0.05 *
	In 6 months	4.02±0.12	3.73±0.11 */†	3.35±0.14 *	3.18±0.10 *

Notes: * – p<0.05 compared to the initial level in the corresponding subgroup (before treatment);
† – compare to the control (dental-healthy adolescents without GIT comorbidity).

Table 2. The frequency of plating of normal microflora transient representatives (continuous index, %) out of gingival mucosa of the adolescents with GCG during treatment

Patients subgroups		*S. epidermidis*	*Stomatococcus mucilaginosus*	*Neisseria sp.*	*Corynebacterium sp.*
Control		30.0±3.3	45.0±3.6	15.0±2.6	10.0±2.1
GCG + Gastroduodenitis					
1 A	Before treatment	42.1±2.6 †	47.4±2.6	21.1±2.2 †	10.5±1.6
	After treatment	26.3±2.3 *	52.6±2.6 */†	15.8±1.9 *	5.3±1.2 */†
	In 6 months	42.1±2.6 †	47.4±2.6	21.1±2.2 †	15.8±1.9
1 B	Before treatment	63.2±2.5 †	57.9±2.6 †	26.3±2.3 †	21.1±2.2 †
	After treatment	42.1±2.6 */†	31.6±2.5 */†	10.5±1.6 *	10.5±1.6 *
	In 6 months	52.6±2.6 */†	42.1±2.6	26.3±2.3 †	10.5±1.6 *
GCG					
2 A	Before treatment	53.9±3.8 †	53.9±3.8 †	23.1±3.2 †	15.4±2.8
	After treatment	30.8±3.6 *	46.2±3.8 *	30.8±3.6 */†	23.1±3.2 */†
	In 6 months	61.5±3.7 */†	53.9±3.8 †	15.4±2.8 *	30.8±3.6 */†
2 B	Before treatment	41.7±4.1 †	50.0±4.2 †	16.7±3.1	25.0±3.6 †
	After treatment	25.0±3.6 *	25.0±3.6 */†	8.3±2.3 */†	8.3±2.3 *
	In 6 months	41.7±4.1 †	58.3±4.1 †	8.3±2.3 */†	33.3±3.9 */†

Notes: * – p<0.05 compared to the initial level in the corresponding subgroup (before treatment); † – compare to the control (dental-healthy adolescents without GIT comorbidity).

In the patients of subgroups 1A and 2A, the new therapeutic complex allowed achieving a steady decrease in the massiveness and colonization frequency of gum mucosa by pathogenic aerobic microflora: β-*hemolytic streptococcus S. pyogenes, S. aureus,* golden staphylococcus, and Candida genus yeast fungi.

In the adolescents with GCG and underlying gastroduodenitis (subgroup 1A) immediately after treatment, the fact of a full disappearance of golden staphylococci from germ microbiocenosis was established. In six months after the treatment, it was revealed in only one patient (CI 5.3±1.2% with a minimum PL of 3.0 lg CFU/ml). At the same time, a significant decrease in β-hemolytic streptococcus gum (CI 10.5±1.6%, p<0.05) was also evidenced compared to the initial levels as well as in yeast-like fungi of genus Candida (CI 5.3±1.2%, p<0.05).

In the patients with GCG without concomitant gastroduodenal disease (subgroup 2A), the suggested therapeutic complex promoted to the disappearance of yeast fungus from gum mucus, but this event was not longlasting. In 6 months in 2 patients (5.4±2.3%) the colonization of gums by candidiasis was present again with an average PR 3.35±0.14 lg CFU/ml. In the subgroup 1B (GCG in combination with gastroduodenitis), the traditional therapeutic complex did not allow achieving a significant decrease in gonadal colonization rates by pathogenic microbiota.

Discussion

The attained results of clinical examination of the adolescents of the main group, who underwent the suggested comprehensive treatment of catarrhal gingivitis, proved a more significant positive dynamics of the indices compared to the adolescent of the comparison group. Thus, the developed new therapeutic complex for treatment of adolescents with GCG and underlying concomitant gastroduodenitis proved a significant corrective effect on the nature of microbiocenosis gums. This allowed achieving a stable normalizing effect on the resident and transient normoflora and ensured a decrease in the proportion of pathogenic aerobic microorganisms in oral microbiocenoses of the examined adolescents.

The analysis of the conducted microbiological study prove that the microbiocenosis of gum mucus is caused by increased colonization by representatives of resident α-hemolytic streptococci (p<0.01) and transient microflora of oral cavity: epidermal staphylococcus (p<0.05), stomatococcus (p<0.05) and corynebacteria (diphtheroids) (p<0.05), and also is accompanied by a higher level of colonization of the affected areas by *Staphylococcus aureus* (p<0.05), *β-hemolytic streptococci* (p<0.05) and yeast-like fungi of the genus Candida, which confirms the established present concept of inflammatory diseases with underlying periodontal disease, which deals with the microbial factor triggering

inflammation in cases of periodontal disease, in children and adolescents [8,14].

Therefore, the detail study of the changes of microbiocenosis in adolescents, to be precise at the initial stage of inflammatory development, is urgent; much attention should be paid to the constant factors that may contribute to a long-term development of the disease with its transition to a more severe degree as well as occurrence of relapses. In addition, the results of microbiological examination can be a diagnostic criterion for the effectiveness of treatment and prognostication of the subsequent course of the inflammatory process in gums. The dynamics of clinical parameters and their changes in microbial associations in the course of treatment confirms the necessity of a repeated course of treatment for the adolescents with concomitant somatic pathology and underlying chronic gastroduodenitis, as well as without any somatic pathology, in order to obtain stable results and prevent recurrence.

The attained results are important for dental practice as well as for general pediatric practice, since the oral cavity is the initial part of gastrointestinal tract, periodontal tissues can be a reservoir for opportunistic and pathogenic microflora, and therefore cause not only periodontal tissue diseases but reinsert the lower sections of gastrointestinal tract and affect the course and results of treatment of common somatic diseases.

Consequently, the results prove the need for the development of the scheme of treatment and prophylactic complex of professional oral hygiene, hygiene training, monitoring the stable motivation to comply with individual oral hygiene in addition to drugs aimed at various pathogenesis links of the disease, as is also evidenced by other researchers [18].

Conclusions

The attained results allow us drawing a conclusion that a high clinical efficacy of the suggested complex, which contributes to a prolonged positive dynamics and stable changes in periodontal tissues at the early period of treatment, which is confirmed by positive changes in the indexes.

The therapeutic and prophylactic complex for treatment of the adolescents with GCG has a steady corrective effect on normal gum microflora (the composition of α-hemolytic streptococci, their quantitative characteristics of colonization, population level (PL) and index of constancy (Continuous Index, CI) stomatococci, non-toxic, and diphteroids).

The suggested treatment and prophylaxis complex reduce the massivity and colonization frequency of gum mucosa by pathogenic aerobic microflora (β-hemolytic streptococci, *Staphylococcus aureus*, and yeast-like fungi of genus Candida).

ДИНАМІКА ЗМІН МІКРОБІОЦЕНОЗУ ТКАНИН ПАРОДОНТА ЗА УМОВ КОМПЛЕКСНОГО ЛІКУВАННЯ У ПІДЛІТКІВ З КАТАРАЛЬНИМ ГІНГІВІТОМ ТА ХРОНІЧНИМ ГАСТРОДУОДЕНІТОМ

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ІВАНО-ФРАНКІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ, ІВАНО-ФРАНКІВСЬК, УКРАЇНА

Вступ. *Якісні та кількісні зміни мікрофлори, нехтування гігієною ротової порожнини та ослаблення місцевого і системного імунітету, які частіше зустрічаються на фоні соматичної патології, відіграють ключову роль у етіології та патогенезі захворювань тканин пародонту.*

Мета дослідження *встановити клінічну та мікробіологічну ефективність застосування запропонованого лікувально-профілактичного комплексу у підлітків з катаральним гінгівітом та хронічним гастродуоденітом.*

Методи. *Обстежено 38 підлітків віком 12-18 років з катаральним гінгівітом та хронічним гастродуоденітом (основна група). У групу порівняння включено 25 підлітків з катаральним гінгівітом без наявності супутньої соматичної патології.*

Результати. *Встановлено, що застосування запропонованої нами для лікування комбінації (засіб рослинного походження з антимікробними властивостями, застосування дентального гелю з метронідазолом та хлоргексидином разом з капсулами пробіотика) значно перевищує за своєю*

ефективністю традиційні методи терапії. Відмічено значний позитивний вплив на показники мікробіоценозу тканини пародонту у з катаральним гінгівітом та хронічним гастродуоденітом.

Висновки. *Запропонований лікувально-профілактичний комплекс значно зменшує колонізацію патогенної аеробної флори (Стрептокок групи В, золотистий стафілокок, гриби роду Candida) ротової порожнини.*

КЛЮЧОВІ СЛОВА: **катаральний гінгівіт; хронічний гастродуоденіт; підлітки; мікробіоценоз; лікувальний комплекс**.

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INFRASTRUCTURE, RESOURCES, SERVICES EVALUATION AND GAP ANALYSIS OF INTEGRATED MATERNAL AND CHILD DEVELOPMENT SERVICES IN INDIA

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Background. *Integrated Child Development Services (ICDS) is an Indian community-centric government program organized under Anganwadi centres catering to supplementary nutrition, health and preschool education, primary healthcare, growth monitoring and counselling the children under six years old along with their mothers. It is the world's largest outreach program in a developing country covering a population of 1.35 billion; the variations in service delivery were analysed involving cross-sectional rural and urban Anganwadi centers in New Delhi.*

Methods. *Data were collected by assessment of children and mothers, interview of Anganwadi workers and observation of service delivery parameters and conduction of activities. Infrastructural, beneficiaries, services and content were evaluated by a suitable pre-tested questionnaire based on the National Institute of Public Cooperation and Child Development (NIPCCD) evaluation proforma. The data was analysed by a descriptive statistics.*

Results. *Gaps were found in respect of infrastructure, resources, health and nutrition facilities especially at rural Anganwadi centre which was inadequate in terms of implementation of nutrition and health program, supplementary nutrition, preschool education and nutrition rehabilitation centre for existing beneficiaries. Both Anganwadi centres were not catering for new WHO growth standards and adolescent health.*

Conclusions. *Gaps found in respect of infrastructure, resources, health and nutrition facilities can affect performance of ICDS program and the services delivered by Anganwadi centres, which need a boost. Both urban and rural centres have a direct opportunity towards delivering adolescent health program focusing on nutrition and education of girls prior to their pregnancy, and adoption of new WHO growth standards.*

KEY WORDS: **ICDS, Anganwadi centre, nutrition, maternal health, growth charts.**

Introduction

Integrated Child Development Services (ICDS) is a medico-social, gender-neutral, community-centric philanthropic program organized under rural courtyard shelters known as Anganwadi centres, catering to supplementary nutrition, nutrition and health education, preschool non-formal education, primary healthcare, immunization and growth monitoring of the children under six years old along with their mothers [1]. ICDS was launched in 1975 in accordance with the National Policy for Children in 33 places throughout India complemented by 13.3 lakh Anganwadi centres across the country. Organization of ICDS is implemented at five different levels, i.e. Central Level, State/Union Territory Level, District Level, Block Level and Village Level covering rural areas, tribal areas and urban slums to serve the extremely underprivileged communities of backward and remote areas of the country. Each Anganwadi centre covers 40-42 children from 0-6 years old and 20-25 pregnant and lactating mothers delivering services right at the doorsteps of the beneficiaries to ensure their maximum participation [2]. The ICDS team comprises Child Development Project Officers, District Program Officers, and auxiliary staff.

Being the world's largest outreach program in targeting infants and children below six years old, expectant and nursing mothers, the ICDS has generated interest worldwide among academicians, planners, policy makers, administrators and those responsible for its implementation [3]. Given its effectiveness over the last few decades, the Government of India is committed towards ensuring universal availa-

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bility of ICDS. The ICDS program at Anganwadi centres that are meant to adhere to certain laid-down standards given by the government for various infrastructural, resource and service parameters, for optimal functioning of the unit. The resources/infrastructure available on ground and services delivered vary widely. These variations account for the difference in standards of service-delivery, which in-turn might make a difference in overall effectiveness of ICDS. Thus, the availability of infrastructure, human resources and delivery of services should be studied and compared against the laid-down standards, for ascertaining gap analysis. It is obvious that bridging the gaps will help improve the program and offset malnutrition. Various evaluations of ICDS have revealed outcomes requiring improvement in the nutritional status of children which were falling short of expectations. This cross-sectional study has evaluated and analysed the gap, with respect to infrastructure, resources and ICDS services *vis a vis* the laid-down standards, at rural and urban Anganwadi centres in New Delhi.

Methods

A cross-sectional evaluation study was carried out among two Anganwadi centres in New Delhi. The data were collected by assessment of children and mothers, interview of Anganwadi workers and observation of service delivery parameters and conduction of activities. Infrastructural assessment of ICDS program via Anganwadi centres was done by observing building infrastructure, floor, roof, electric-supply, availability of kitchen or washroom, kitchen-utilities, drinking-water, preschool education material, guidebook, growth charts, proportion of malnourished children, supplementary nutrition to pregnant females, counselling activities towards nutrition, health, immunization, maintenance of records about beneficiaries and services availed. Suitable pretested and adequately modified questionnaire based on the National Institute of Public Cooperation and Child Development (NIPCCD) evaluation proforma was designed to interview the Anganwadi worker and the beneficiaries (mothers/children). Data was analysed under descriptive statistics.

Results

Both Anganwadi centres rented cemented building with adequate indoor space, child-friendly unisex toilets, electricity supply and water. Both Anganwadi centres were provided with hot cooked food containing a varied combination of pulses, cereals, oil, vegetables and sugar as well as take-home rations. The urban and rural Anganwadi centres involved 11 and 12 pregnant women, seven lactating women each, 35 and 36 children aged from 6 months to 3 years, as well as 30 and 18 children aged 3-6 years respectively. No children were found to be underweight. No adolescent girls were beneficiaries at either Anganwadi centres. Pregnant women were counselled towards utilisation of key services i.e. antenatal care, iron and folic-acid supplementation, adequate extra-care from family and rest during pregnancy. There was no interruption in supplementary nutrition in past six months. Supplementary nutrition program in urban Anganwadi centre was more effectively used by beneficiaries as compared to rural Anganwadi centre, where quality was neither satisfactory nor accepted by beneficiaries, while it was adequately accepted in the urban centre. In rural Anganwadi centre, Nutrition and Health Education program was not followed. As for infrastructural facilities, both Anganwqadi centres had good electricity supply with electric points above 5 feet from ground that was safe for children. Direct tap-water was stored in covered containers as per standards however there was no provision of either water-filters or reverse osmosis plants.

New WHO growth standards were not implemented in both Anganwadi centres, but weighing, plotting, interpretation and counselling of mothers were accurately performed and they were provided with WHO Growth chart. Early childhood care and education program was conducted in both Anganwadi centres every month as per laid-down standards. Urban Anganwadi centre was adequately provided with preschool education materials such as time-table, guide-book for story-telling, counting numbers, free conversation sessions to enable speaking and organized small-activities related to fine muscle coordination and development such as painting, drawing, threading and matching colour, reading simple words, writing alphabets words, distinguish objects, recognise pictures etc. The rural Anganwadi centre was also provided with preschool education materials but not adequate ones as per laid-down standards.

Both Anganwadi centres were undertaking routine health check-up. It was noted that in the urban Anganwadi centre, the frequency of health check-up was monthly for children aged

1-3 years, quarterly for children aged 3-6 years, and four check-ups were provided to pregnant females starting in first trimester. In rural Anganwadi centre, pregnant females were seen only twice starting in second-trimester. Both Anganwadi centres didn't include adolescent girls for health check-up. In both Anganwadi centres, immunization program was running effectively. Iron and Vitamin A supplementation were provided to children and pregnant women under the ICDS program.

Discussion

Recognizing the need for early intervention to ensure the development of a young child's body, mind, and intellect to its maximum potential, the Government of India started the ICDS, a centrally sponsored scheme, which is a step toward responding to the child's needs in a comprehensive and holistic perspective. The national goals of ICDS program are reduction of infant mortality rate to <60/1000, reduction in child mortality rate to <10/1000, and reduction in maternal mortality rate by at least 50%, through a focused health check-up, immunization, deworming, basic treatment of minor illnesses like fever, diarrhoea etc., referral services for severe illness, supplementary feeding, growth monitoring and early childhood day care [4, 5].

Infrastructural evaluation was satisfactory in this study whereas other studies found toilet facilities present only in 57.1% Anganwadi centres [6,7]. The rural Anganwadi centre was lagging the laid-down standards towards nutrition and health program, supplementary nutrition, preschool education and adoption of nutrition rehabilitation centre, which was also found in other rural Anganwadi centres in India by various studies [8, 9, 10, 11]. These services are meant for effective transmission of certain basic health and nutrition messages to enhance the level of awareness of mothers about the child's needs and their capacity for care, protection, and development of the child. Supplementary nutrition is strategic as it not only improves the nutritional level of children and reduces malnutrition, but it also works as an incentive for promoting attendance of children and mothers to participate in the activities of Anganwadi centres, and as such plays a vital role in ICDS program. Inadequacy on these fronts bears a direct connotation towards inadequate service delivery and feto-maternal outcomes as India is mostly a rural country and requires strengthening of rural Anganwadi centres [12, 13, 14, 15].

In current study it was also observed that the new WHO growth standards were not implemented in both Anganwadi centres, but weighing, plotting, interpretation and counselling of mothers were accurately performed, and they were provided with the WHO Growth chart. Similarly, adolescent health is a completely neglected area under the ICDS program which needs to be intensified to ensure nutrition and health of girls prior to pregnancy to enable them to make better choices towards family planning, contraception and perinatal care of themselves and their children [16]. In turn, this will universalize and maximise the concept development of ICDS program in context and content; and further capacity building initiatives amongst beneficiaries for better families, better management of Anganwadi centres and a sustainably better future in a tropical developing country withholding a population of 1.35 billion [17, 18, 19].

Conclusions

Gaps found in respect of infrastructure, resources, health and nutrition facilities can directly affect the performance of ICDS program and the services delivered by the Anganwadi centres. There is a felt opportunity to boost the service delivery ecosystem of rural Anganwadi centre in term of implementation of nutrition and health program, supplementary nutrition, preschool education and nutrition rehabilitation centre for existing beneficiaries. Both urban and rural centres have a direct opportunity towards delivering adolescent health program focusing on nutrition and education of girls prior to their pregnancy, in order to maximise the potential of ICDS program and universalise the acceptance and availability of ICDS. There is a scope of improving upon adoption of new WHO growth standards.

ІНТЕГРОВАНІ ЦЕНТРИ МАТЕРІ І ДИТИНИ В ІНДІЇ: ІНФРАСТРУКТУРА, РЕСУРСИ, ОЦІНКА ДІЯЛЬНОСТІ ТА ПРОГАЛИНИ У РОБОТІ

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Вступ. *Об'єднані центри дитячого розвитку (ICDS) – це державна соціально-орієнтована програма у Індії під егідою Анганваді центрів, яка забезпечує додаткове харчування, санітарно-гігієнічну просвітницьку діяльність та дошкільну освіту, надання первинної медичної допомоги, моніторинг та консультування дітей віком до шести років разом з їхніми матерями. Це найбільша у світі за охопленням (коло 1,35 мільйонів населення) програма у країнах, які розвиваються.*

Мета роботи – *проаналізувати діяльність Анганваді центрів у міській та сільській місцевостях Нью Делі.*

Методи. *Дані збиралися шляхом опитування матерів та дітей і працівників Анганваді центрів, а також оцінки діяльності центрів. Опитувальники щодо інфраструктури, переваг та наданих центрами послуг базувалися на формі, запропонованій Національним інститутом громадського співробітництва та розвитку дітей. Дані аналізувалися за допомогою описової статистики.*

Результати. *Були знайдені недосконалі моменти у роботі Анганваді центрів, особливо у сільській місцевості, а саме щодо термінів впровадження програм здорового харчування та дошкільної освіти та програм реабілітації. У обох центрах не було запроваджено нові стандарти ВООЗ.*

Висновки. *Виявлені прогалини можуть вплинути на ефективність роботи об'єднаних центрів дитячого розвитку та якість послуг, які надаються у Анганваді центрах. Однак, як міські, так і сільські центри мають можливість здійснювати програми охорони здоров'я підлітків, зосереджуючись на харчуванні та вихованні дівчат до їх вагітності, а також прийняття нових стандартів ВООЗ.*

КЛЮЧОВІ СЛОВА: **ICDS програма; Анганваді центр; харчування; репродуктивне здоров'я жінок; розвиток дітей.**

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MELPHALAN-INDUCED CYTOTOXICITY IN THE BONE MARROW OF RATS BY FLOW CYTOMETRY MEASUREMENTS

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Background. *Bone marrow (BM) that contains hematopoietic cells of various lineages is a sensitive target for a number of cytotoxic agents including chemotherapy drugs.*

Objective. *Flow cytometry (FCM) was chosen to test cytotoxicity in BM of rats, that received melphalan either intravenously (i.v.) or intraperitoneally (i.p.).*

Methods. *One group of rats received melphalan i.v. (3 mg/kg) followed by the BM examination on the 3rd and 7th day after drug administration, whereas another group of animals received this drug i.p. in total doses of 9 and 15 mg/kg followed by the BM examination on the next day after the 3rd and 5th injection of the drug. BM cells were stained with acridine orange and analyzed by FCM. Cytotoxicity was assessed by determining the percentage of total nucleated cells (TNC%) among the whole BM cell population and by determining the percentage of polychromatic erythrocytes (PCE%) among the whole population of enucleated erythrocytes.*

Results. *Regardless of the dose and regimen of melphalan administration, either i.v. or i.p. administered drug caused a significant reduction of TNC%. On the average, the i.p. administered drug resulted in about 2.0-fold decrease of TNC% (P<0.05), while the i.v. administered drug resulted in about 1.3-fold decrease of TNC% (P<0.05). As for enucleated erythrocytes, the i.p. administered drug resulted in about 1.4-fold decrease of PCE% (P<0.05), whereas the i.v. administered drug did not cause any changes in the PCE%.*

Conclusions. *Under these experimental conditions, i.p. administrated melphalan is considerably more cytotoxic than i.v. administered melphalan. This cytotoxic effect is preferentially due to impaired erythropoiesis.*

KEY WORDS: **bone marrow; melphalan; cytotoxicity; flow cytometry; polychromatic erythrocytes; total nucleated cells.**

Introduction

The anti-tumor effect of alkylating chemotherapeutics primarily attributes to their ability to covalently bind DNA via alkyl groups causing intra- and inter-strand crosslinks [1]. Any alkylating drug by induction of DNA lesions can affect the replication of actively proliferating cells [2]. Moreover, an impaired replication or repair of crosslinked DNA is likely to lead to cell death [3]. Although alkylating drugs can specifically target proliferating cells, they are not cell cycle phase-specific, and for this reason, cell death is believed to directly correlate with the dose of the drug [4]. An alkylating agent melphalan (known as interstrand DNA-crosslinker [1]), which is mainly used for treatment of multiple myeloma, ovarian carcinoma, breast cancer, childhood neuroblastoma, and *polycythaemia vera*, however, may cause complications, particularly acute myeloid leukemia in the decade after therapy [5]. Melphalan-treated individuals with an increased level of chromosomal aberrations in the peripheral blood lymphocytes are at risk of developing cancer later in life [5]. In the experimental animals, melphalan induces cancer of various localizations [5], and regardless of the route of administration, it is apparently genotoxic [6-10].

In the present work, flow cytometry (FCM) has been chosen to examine cytotoxicity in the bone marrow (BM) of rats that received melphalan either intravenously (i.v.) or intraperitoneally (i.p.). BM that contains hematopoietic cells of various lineages is a sensitive object of cytotoxic studies. As for the FCM, this technique is indispensable in many areas of biology and medicine not only because of its high-speed analysis, but also because of its ability to accurately discriminate cells of various types. The FCM usually discriminates cells based on their size, intracellular granularity and selective/specific fluorescence labeling [11]. This unique

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advantage of FCM can be applied for the study of cytotoxic effects in BM cells of different lineages and maturation stages. Here we use a simple and reliable FCM approach for the analysis of BM cells stained with acridine orange (AO), a metachromatic dye that simultaneously interacts with DNA and RNA producing at λ=488 nm the dual emission spectra with peaks at 530 nm and 640 nm, respectively [12]. This proposed approach by Criswell et al. [13] allows assessing cytotoxicity particularly in erythropoietic cells based on detection of differences in AO uptake between polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). Redistribution of erythrocytes towards NCE is indicative of cytotoxic effect. Suzuki et al. [14] suggested that the reduced PCE/NCE ratio is most likely caused by elevation of NCE population as a result of mutagen-induced rapid differentiation and multiplication or enucleation of erythroblasts which remained in the BM instead of entering the peripheral blood steam. On the other hand, Von Lebedur and Shcmid [15] claimed that as a result of mutagen-induced partial depletion of the marrow cavities of nucleated blood cell precursors the newly formed erythrocytes can be retained along with inundation with peripheral blood.

Methods

Experimental animals and administration of melphalan

Adult outbred female rats (140-160 g) were taken from the animal house of R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine (Kyiv, Ukraine). Immediately before the i.v. or i.p. injections, 0.6 mg/ml solution of melphalan (Alkeran® produced by GlaxoSmithKline, UK) was prepared by diluting 20 mg/ml solution of this drug dissolved in acidified ethanol (96% ethanol and 12 N HCl mixed in the ratio of 150:1) with saline. One group of animals (n=3) was subjected to i.v. injection (via tail vein) of melphalan of a single dose of 3 mg/kg followed by the BM examination on the 3rd and 7th day after drug administration. Another group of animals (n=3) was subjected to i.p. injections of melphalan every other day with single doses of 3 mg/kg followed by the BM examination on the next day after 3rd and 5th injection of the drug (total doses were 9 and 15 mg/kg, respectively). Since melphalan after i.p. administration is assumed to be less readily delivered to the target tissue than after i.v. administration due to its gradual absorption into blood, this prompted us to increase the total dose of melphalan for delivery via i.p. route. There was also a group of animals that did not receive the drug at all (the intact control, n=3). The study with animals was performed according to the regulations of the Ethics Committee.

BM isolation, specimen processing and fixation

The femur removal and BM isolation procedures were in general performed as proposed [13]. The BM cells were thoroughly flushed from the femur with 4 ml of RPMI-1640 (PharmBiotek, Ukraine) and immediately placed in a refrigerator (+4-6 °C). At this temperature, the BM cells were kept no longer than 1.5 hour before they were resuspended by vortexing and centrifuged at 300× g for 5 min. Specimen processing and fixation procedures were mainly performed according to the protocol [13]. The supernatant was discarded with further washing the cells in 5 ml of PBS using centrifugation at 300× g for 5 min. The supernatant was discarded with further resuspension of cells in 2 ml of PBS by vortexing. Cell aggregates were dissociated by gentle syringing of the suspension through a 21-gauge needle. While vigorous vortexing, 0.2 ml of cell suspension was added to 5 ml of fixative solution: 1% glutaraldehyde (v/v) in PBS with 30 μg/ml of SDS (Merck, Germany). The cells were fixed for 5 min and then centrifuged at 300× g for 5 min. The supernatant was removed with further resuspension of cells in 0.5 ml of PBS.

Fluorescence staining

This procedure with slight modification was performed in accordance with the protocol [13]. Solution A was prepared by dissolving of the following components in 100 ml (final volume) of distilled H_2O: 0.1 ml Triton X-100 (Loba Chemie, Austria), 8 ml 1.0 N HCl, and 0.877 g NaCl. Solution B was prepared by mixing of 37 ml 0.1 M citric acid with 63 ml 0.2 M Na_2HPO_4 (pH 6.0) and adding 0.877 g NaCl, 34 mg EDTA disodium salt (Sigma, USA), and 0.6 ml of acridine orange (AO; Sigma) stock solution (1 mg/ml). The fixed cells (0.2 ml of cell suspension) were added to the mixture of Solutions A and B (0.4 and 1.2 ml, respectively) that was chilled on ice in a 12×75 mm centrifuge tubes. While shaking, the cells were stained on ice for 30 min in the dark. Then they were centrifuged at 300× g for 5 min. After the supernatant was carefully removed, 1 ml of PBS was added to resuspend the cells. Before FCM, cell suspension

was gently syringed through a 21-gauge needle to obtain a suspension of single cells.

FCM analysis

The samples were analyzed using an EPICS XL flow cytometer (Beckman Coulter, USA) equipped with a 15-mW argon-ion laser (488 nm). The forward light scatter (FSC; related to cell size) and side (90°) light scatter (SSC; related to intracellular granularity) signals were collected in a linear mode. The fluorescence signals of DNA- and RNA-bound AO were collected respectively in the green fluorescence channel (FL1) through a 525/10-nm band-pass filter and in the far-red fluorescence channel (FL4) through a 675/10-nm band-pass filter using a logarithmic amplification [13]. The acquisition rate was 500-1000 cells per second. At least 1.0×10^5 events were collected for each sample. The analysis of the data was performed by publicly available software "WinMDI" developed by Dr. J. Trotter (http://www.cyto.purdue.edu/flowcyt/software/Winmdi.htm). The cells were gated on FSC-Height vs. SSC-Height histograms to eliminate debris and aggregates from analysis (not presented here), although microscopic observation showed that their numbers were extremely low. The parameters that were examined are as follows: 1) percentage of total nucleated cells (TNC) of all BM cells, including enucleated cells such as PCE and NCE (this parameter is further denoted as TNC%); 2) percentage of PCE of all enucleated erythrocytes (denoted as PCE%). The reason why $TNC_{\%}$ was also examined is based on the fact that the nucleated erythroid cells are most numerous in the BM, and accordingly, suppressed erythropoiesis may affect TNC%. Thus, the decreased TNC% and PCE% (particularly PCE%) can be indicative of inhibited division and maturation of nucleated erythroid cells, the fact that has been previously reported [13-16]. The populations of TNC, PCE and NCE that demonstrate significant differences in AO uptake were determined on a FL1-Height vs. FL4-Height histogram (Fig. 1A).

Statistical analysis

Probability values with $p<0.05$ were considered statistically significant. The distribution of indices was estimated by using the Shapiro-Wilk Normality Test. The statistical significance of the differences between the means was assessed by the Mann-Whitney-test and ANOVA-test using Origin 7.5 software (OriginLab Corporation, USA).

Results

Regardless of the dose and regimen of melphalan administration, i.v. delivered melphalan did not cause any significant changes in the PCE%, whereas i.p. delivered drug on the average resulted in about 1.4-fold decrease of PCE% ($p<0.05$, compared with the control; Fig. 2).

As for TNC, melphalan administered either i.v. or i.p. resulted in a significant decrease of

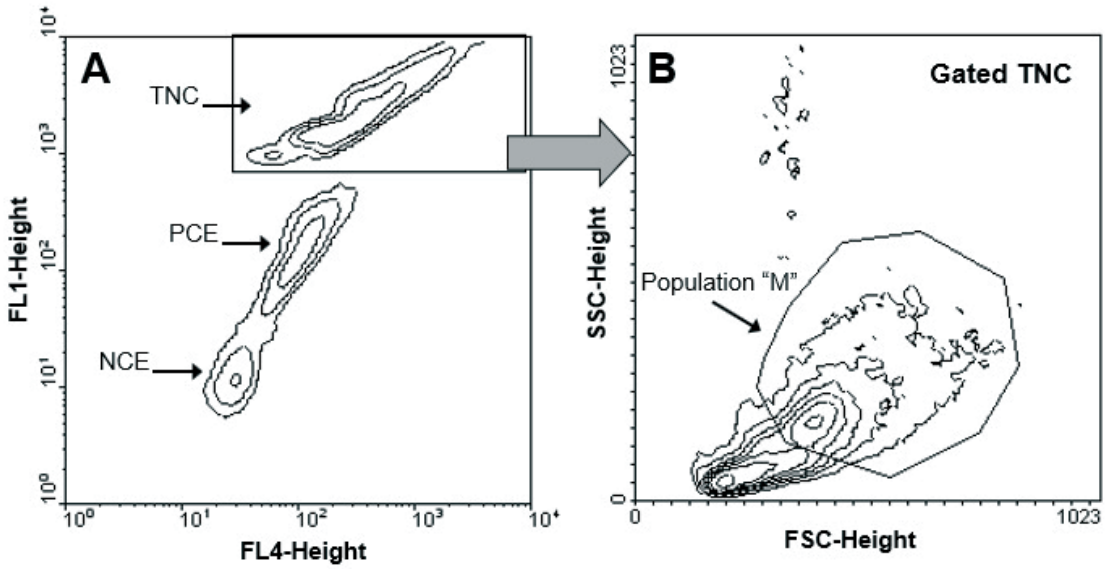

Fig. 1A. Example of FCM determination of TNC, PCE, NCE populations in AO-stained unfractionated BM cells (BM cells were isolated from the femur of the control intact rat).
Fig. 1B. Population of TNC gated on a FL1-Height vs. FL4-Height histogram (framed by the rectangular window; panel A) is shown on a FSC-Height vs. SSC-Height histogram to analyze the population 'M' comprised of the vast majority of myeloid cells.

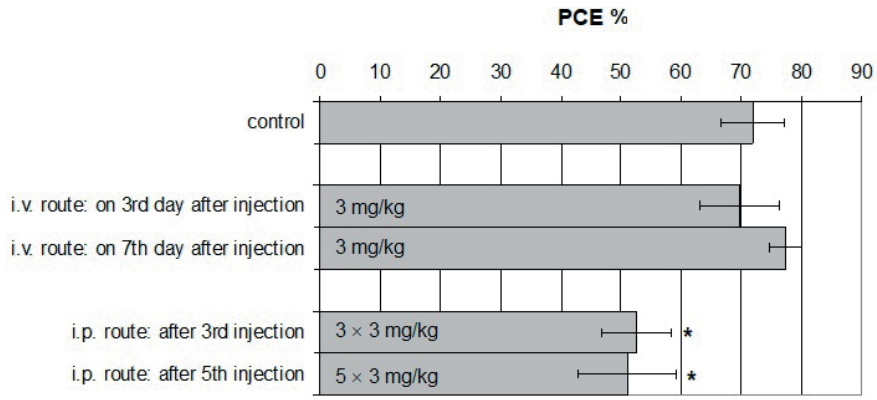

Fig. 2. Effect of variously administered melphalan on the PCE%. The data presented are the mean ± standard error of the mean. Each group consists of three animals. Asterisks (*) show significant differences if compared with the control ($p<0.05$).

TNC% ($p<0.05$, compared with the control Fig. 3). On the average, after i.p. and i.v. drug delivery there was a 2.0-fold and 1.3-fold decrease of TNC%, respectively (Fig. 3). Obviously, melphalan after several i.p. administrations (3×3 mg/kg or 5×3 mg/kg) was more cytotoxic than after a single i.v. administration (3 mg/kg). However, increasing the dose of i.p. injected melphalan up to 5×3 mg/kg did not result in more significant cytotoxic effect. Perhaps, at lower dose of this drug (3×3 mg/kg) the maximal effect could be reached. As for the i.v. delivery of melphalan, we did expect that this route of drug administration would be more efficient in terms of causing cytotoxicity in the BM. That is why a single minimal dose of melphalan (3 mg/kg) was chosen for this route of delivery.

Although the aforementioned findings seem to be indicative of suppressed proliferation of erythroid cells, particularly in case of i.p. administered melphalan, it cannot be certainly claimed that proliferation of myeloid cells remains unaffected. Since myeloid cells as well as erythroid cells are numerous in the BM [17], one can expect that the drug-induced suppression in proliferation of these cells may also contribute to significant fluctuations of TNC%. To address this issue, we were able to identify within TNC the population of cells (population 'M'; Fig. 1B), the vast majority of which are likely to be myeloid. This assumption is simply based on the evidence that they are generally large with a specific intracellular granularity [17-19]. In FCM, cell size usually correlates with the FSC, while intracellular granularity correlates with the SSC [11]. The percentage of population 'M' of the whole TNC population (denoted as population 'M'%) was similar to that revealed microscopically by the classic morphology-based evaluation (date not shown). Notably, i.p. administered melphalan on the average caused a 1.3-fold increase of the portion of population "M" (Fig. 4) with a concordant decrease of PCE% (Fig. 2). However, together with the fact that the i.p. delivered drug resulted in a 2-fold decrease of TNC% (Fig. 3) one can assume that granulopoiesis is likely be affected but certainly to a lesser extent then erythropoiesis. As for the i.v. delivered

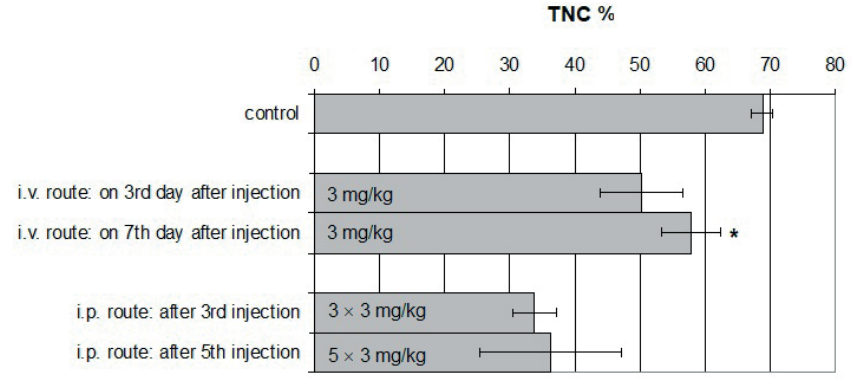

Fig. 3. Effect of variously administered melphalan on the TNC%. The data presented are the mean ± standard error of the mean. Each group consists of three animals. Asterisk (*) shows significant difference if compared with the control ($p<0.05$).

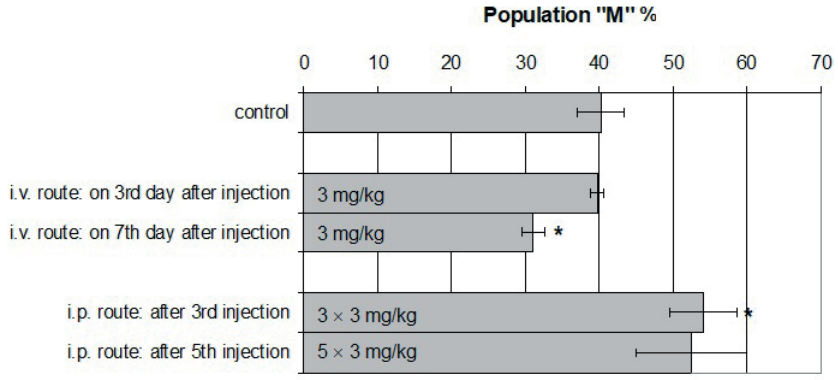

Fig. 4. Effect of variously administered melphalan on population 'M'%. The data presented are the mean ± standard error of the mean. Each group consists of three animals. Asterisks (*) show significant differences if compared with the control ($p<0.05$).

drug, there was a 1.3-fold decrease of population "M"%, but only on the 7th day of its injection ($p<0.05$, compared with the control; Fig. 4). This decrease of population 'M'% was accompanied by a slight increase of PCE% on the same day after i.v. drug administration (Fig. 2). Perhaps, on this day (the 7th day) after i.v. drug administration the erythroid cells recovered faster than myeloid cells.

Discussion

Regrettably, there is lack of reports concerning cytotoxic or genotoxic effects in the BM of rats caused by melphalan delivered either i.v. or i.p. Instead, it was reported that the rats that intramuscularly received this alkylating agent of a single dose of 1 mg/kg developed a transient but significant increase of chromosomal aberrations in BM cells peaking on the next day after the drug administration (but there were almost no aberrations on the second day after the drug administration, similar to the control), while increasing the dose up to 10 mg/kg led to the absence of mitotic figures, which is indicative of significant BM suppression [6].

Thus based on the results of our research, one can assume that erythropoiesis is more readily affected by melphalan than granulopoiesis was, but erythropoiesis seemed to be recovered faster than granulopoiesis. Apparent erythropoietic cytotoxicity in the BM of rats was shown to be caused by another alkylating drug cyclophosphamide that at the range of doses 5-40 mg/kg resulted in a significant increase of myeloid/erythroid ratios on the second day after i.p. drug delivery [20, 21].

Erythroid cells were shown to be very sensitive with respect to ionizing radiation (IR), DNA damaging agent of a physical origin [22, 23]. For example, as a result of a whole-body irradiation of rats with the dose of X-rays about 7.0 Gy (LD_{50}) the erythroid cells appeared to be significantly more sensitive than the myeloid cells; however, erythropoiesis began recovering much earlier than granulopoiesis (obvious regeneration was first clearly observed on the 12th day after irradiation as evidenced by areas of erythropoiesis) [21]. Notably, the cytotoxic effect of any alkylating agent is referred to as 'radiomimetic' because IR and alkylating agents are similar in terms of inducing cell death mechanisms (both of them induce the mitotic catastrophe) [24, 25]. Interestingly, as for melphalan, its dose-response relationship resembles that for IR as evidenced by the shape of the survival curve [24]. A series of studies has been initiated towards tackling melphalan-induced BM suppression [26, 27], and, in this regard, monitoring of BM recovery by FCM could be helpful as well.

Conclusions

Under the present experimental conditions, i.p. administrated melphalan is considerably more cytotoxic than i.v. administered melphalan, and this effect is preferentially due to impaired erythropoiesis. Granulopoiesis is less readily affected by the melphalan than erythropoiesis, but on the other hand, granulopoiesis, if affected, is slower recovering than erythropoiesis. It is expected that the FCM findings of this study could be helpful for experimental oncologists, who design experiments on antitumor effects of melphalan with less side effects.

ОЦІНКА МЕЛФАЛАН-ІНДУКОВАНОЇ ЦИТОТОКСИЧНОСТІ НА КЛІТИНИ КІСТКОВОГО МОЗКУ ЩУРІВ ЗА ДАНИМИ ПРОТОЧНОЇ ЦИТОМЕТРІЇ

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Вступ. *Кістковий мозок, як основний орган гемопоезу, особливо чутливий до впливу цілого ряду цитотоксичних протипухлинних лікарських засобів.*

Мета роботи: *за допомогою проточної цитометрії встановити прояви цитотоксичності на клітини кісткового мозку щурів за умов довенного та інтраперитонеального введення мелфалану.*

Методи. *Кістковий мозок щурів досліджували на 3-ю та 7-у доби після довенного введення мелфалану в дозі 3 мг/кг; а також на наступний день після 3-ї та 5-ї ін'єкцій препарату при його інтраперитонеальному застосуванні (при досягненні кумулятивної дози 9 та 15 мг/кг). Клітини кісткового мозку забарвлювали акридиновим помаранчевим та аналізували за допомогою проточної цитометрії. Цитотоксичність оцінювали за відсотком загальної кількості ядерних клітин (ЯК%), а також за відсотком поліхроматофільних еритроцитів (ПХЕ%) у складі всіх без'ядерних еритроцитів.*

Результати. *Незважаючи на шлях введення та обрану дозу, мелфалан викликав достовірне зниження ЯК%. В середньому, показник ЯК% знижувався у 2 рази при інтраперитонеальному введенні ($p<0.05$) та в 1.3 рази – при довенному ($p<0.05$). Стосовно без'ядерних еритроцитів, ПХЕ% знижувався в 1.4 рази при інтраперитонеальнему введенні ($p<0.05$), тоді як при довенному введенні цей показник залишався без змін.*

Висновки. *В даних експериментальних умовах, на відміну від довенного застосування, інтраперитонеально введений мелфалан виявився більш цитотоксичним, переважно за рахунок порушень еритропоезу.*

КЛЮЧОВІ СЛОВА: **кістковий мозок; мелфалан; цитотоксичність; проточна цитометрія; поліхроматофільні еритроцити; ядерні клітини.**

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GENDER AND AGE ASPECTS OF BIOENERGETICS PROCESSES IN EXPERIMENTAL PASSIVE TOBACCO SMOKING AND MONOSODIUM GLUTAMATE ADMINISTRATION

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Background. *Active smoking and exposure to passive smoke are responsible for more than 5 million deaths each year. At the same time, a characteristic feature of present food technologies is the use of food additives that are not always safe for human health, such as monosodium glutamate (MSG).*

Objective. *The aim of the research was to determine the changes in mitochondrial enzymes activity in rats in case of passive tobacco smoke combined with prolonged administration of MSG in the sex and age aspects.*

Methods. *The evaluation of bioenergetics processes in the mitochondria of circulating neutrophils was carried out using succinate dehydrogenase (SDG) and cytochrome oxidase (CO) activity.*

Results. *Passive tobacco smoke combined with the MSG administration in mature male-rats is accompanied by a significant inhibition of bioenergetics processes, as evidenced by a decrease in succinate dehydrogenase activity by 47.1% ($p<0.001$) compared to the intact animals, which is by 27.9% ($p<0.001$) below this index in case of the isolated effect of tobacco smoke and reduction of cytochrome oxidase activity by 27.5% ($p<0.001$) compared to the control group.*

Conclusions. *Thus, the findings suggest that low dose intake of monosodium glutamate enhances the ability of tobacco smoke to disrupt the cell's bioenergetics processes by affecting the respiratory chain function and generation of ATP. Therefore, it is advisable to investigate the established toxic doses of E621, as well as to study the molecular mechanisms of the 'safe' (allowed) doses of MSG effect on a living organism.*

KEY WORDS: **passive tobacco smoking; monosodium glutamate; cytochrome oxidase; succinate dehydrogenase.**

Introduction

The WHO estimates that in 2015 there was about 1.1 billion adult smokers worldwide, representing nearly a quarter (22%) of the global adult population. Number of women of reproductive age, who smoke, is also increasing. It is expected that by 2025 more than 500 million women will be smokers, accounting for about 20% of the global female population [1-2]. According to Solomenchuk T. M., in Ukraine the prevalence of smoking among women has tripled over the past 30 years [3].

Over 6 million people die from tobacco each year. More than 5 million of those deaths are the result of direct tobacco use while more than 600,000 are the result of nonsmokers being exposed to secondhand smoke. Secondhand smoke is a major health hazard, especially for infants and children. In the United States, it has been estimated that 43% of children aged from 2 months to 11 years live in a home with at least one person that smokes. The prevalence of passive infant smoking was reported to be around 40% in Europe as well [4].

At the same time, the characteristic feature of present food technologies is the use of food additives. One of the most common food additives in Ukraine as well as in Europe is monosodium glutamate (MSG). Encoded E621, it is a food additive from a group of flavor enhancers, used in a wide range of foods, such as soups, sauces, mixed condiments, chips, meat products, and puddings. Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals [5].

An important role in the implementation of toxic action of xenobiotics is the violation of the energy supply in a cell. The most toxic metabolites, as well as products of their initiated lipoperoxidation disturb oxidation of substrates with dehydrogenases, transport electrons

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along the respiratory chain, causing the uncoupling of mitochondrial oxidative phosphorylation. Irreversible disorders in the structure and functioning of mitochondria, caused by the action of excessive amounts of reactive oxygen species (ROS), cause displacement of energy metabolism towards increasing the intensity of glycolysis and inhibition of oxidative phosphorylation [6].

The aim of this investigation was to determine the changes of mitochondrial enzymes activity in rats in case of secondhand tobacco smoke combined with prolonged administration of monosodium glutamate in the sex and age aspects.

Methods

Experimental studies were conducted on 32 inbred mature white male rats weighing 180-200 g, 32 mature nonlinear white female rats weighing 180-200 g and 32 nonlinear immature white male rats weighing 60-80 g.

Each group of animals was divided into four subgroups: the 1st – the intact rats (n=8); the 2nd – the rats with modeled passive tobacco smoking (n=8); the 3rd – the rats, which were injected with monosodium glutamate (n=8); the 4th – the rats with modeled passive tobacco smoking combined with monosodium glutamate injection (n=8).

The investigations were conducted following the general rules and regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethics on Animal Experimentation (Kyiv, 2001).

The rats of the second experimental group were exposed to tobacco smoke for 30 days. The model of the passive tobacco smoking was created by means of airtight chamber volume of 30 liters that allows fumigating animals, which could move freely [7]. Tobacco smoke, formed by smoking of 2 cigarettes Prima sribna (chervona) (containing 0.8 mg of nicotine and 10 mg of tar), was delivered into it through openings in the chamber. Four animals were simultaneously in the chamber for 30 minutes. The third group was administered MSG diluted in a distilled water at a dose of 30 mg/kg body weight (corresponds dose 2 g per day in humans) for 30 days via intragastrical tube [8]. MSG was produced by Sigma-Aldrich (USA). Rats of the fourth group were exposed to tobacco smoke and MSG administration in combination for 30 days.

On the 31-st day the experimental animals were euthanized under thiopental anesthesia and the whole blood was used for further investigation. The population of neutrophils was obtained by whole blood centrifugation at double density gradient 1.077 and 1.093 of Ficoll-Urografin. After 40 minutes of centrifugation at 4 °C and the speed of 1500 rpm, two interphases were formed. The upper interphase (on the border of plasma – Ficoll-Urografin density 1.077) consisted of mononuclear cells: 80% lymphocytes, 15-18% monocytes and 2-3% granulocytes. Lower interphase (on the border of solutions gradient density 1.077-1.092) was the population of neutrophils. The number of viable cells presented in a cell suspension was 98-99% (Trypan blue exclusion test).

The analysis of cell samples to determine neutrophils with ROS (hydrogen peroxide) overproduction was evaluated by the flow laser cytometry method on flow cytometer Epics XL (Beckman Coulter, USA), using 2.7-dichlorodihydrofluorescein diacetate. The value of the studied parameter was expressed as a percentage (ratio of cells with ROS overproduction to general cell count×100 %).

The evaluation of bioenergetics processes in the mitochondria of neutrophils was carried out using succinate dehydrogenase activity (SDG), which was studied by the reaction of the reduction of potassium ferricyanide, which solution had a yellow color, to colorless potassium ferrocyanide by succinate under the influence of SDG and cytochrome oxidase activity (CO) by the oxidation reaction of dimethyl-n-phenylenediamine. All spectrophotometric measurements were made on a SF-46 spectrophotometer.

Statistical processing of digital data was carried out using the software Excel (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA). The distribution of data was analyzed according to assessment of normality by Kolmogorov-Smirnov criterion. The obtained values had a normal distribution, so the difference between the groups was analyzed using the Student's t-criterion. All data were presented as M (mean)±m (standard error). A probability level (p value) of less than 0.05 was considered to be statistically significant. The influence of factors on the indices was determined using the one-factor dispersion analysis (ANOVA). The linkage between the studied indices was established on the basis of the results of the correlation analysis using the Pearson correlation coefficient.

Results

Our studies have proved that succinate dehydrogenase activity in leukocytes mitochondria in the mature male-rats under passive tobacco smoking significantly decreases by 26.6% compare to the control group (Table 1). Passive tobacco smoking combined with the monosodium glutamate injection is accompanied by an even greater decrease in succinate dehydrogenase activity (by 47.1%, p<0.001) vs. the control group, which is 27.9% (p<0.001) below this indicator, providing isolated effect of tobacco smoke. In this case, the prolonged administration of glutamate monosodium results in a less significant decrease in succinate dehydrogenase activity (by 17.2%, p<0.02) compared to the control rats.

In the mature female rats, passive tobacco smoking is accompanied with the decrease in succinate dehydrogenase activity in blood mitochondria of leukocytes by 39.8% (p<0.001) compared to the control group. Passive tobacco smoking combined with the monosodium glutamate injection is accompanied by an even greater decrease in succinate dehydrogenase activity (by 62.1%, p<0.001) compared to the control group, which is 37.1% (p<0.001) below this indicator, provided isolated effect of tobacco smoke. At the same time, prolonged administration of monosodium glutamate results in a decrease in succinate dehydrogenase activity by only 9.6% (p<0.05) compared to the control rats.

In the gender aspect, the intensity of changes in succinate dehydrogenase activity exceeded the rates of the mature male-rats in case of passive tobacco smoking by 13.2%, in case of its combination with the monosodium

Table 1. Influence of passive tobacco smoking and monosodium glutamate on the energy processes in neutrophils mitochondria of rats (M±m, n=8)

Indicator	Groups of experimental animals			
	Intact	Passive tobacco smoking	Monosodium glutamate	Passive tobacco smoking+ monosodium glutamate
Mature male-rats				
SDG, nmol/(mg×min)	2.44±0.09	1.79±0.09 p_1<0.001	2.02±0.10 p_1<0.02	1.29±0.07 p_1<0.001 p_2<0.001 p_3<0.001
CO, nmol/(mg×min)	2.00±0.07	1.69±0.09 p_1<0.05	1.79±0.05 p_1<0.05	1.45±0.07 p_1<0.001 p_2>0.05 p_3<0.01
Mature female-rats				
SDG, nmol/(mg×min)	2.51±0.08	1.51±0.07 p_1<0.001	2.27±0.06 p_1<0.05	0.95±0.05 p_1<0.001 p_2<0.001 p_3<0.001
CO, nmol/(mg×min)	2.07±0.09	1.57±0,06 p_1<0.001	2.03±0.08 p_1>0.05	1.29±0.04 p_1<0.001 p_2<0.05 p_3<0.001
Immature male-rats				
SDG, nmol/(mg×min)	2.91±0.08	1.66±0.11 p_1<0.001	2.12±0.08 p_1<0.001	0.85±0.04 p_1<0.001 p_2<0.001 p_3<0.001
CO, nmol/(mg×min)	2.19±0.07	1.49±0.04 p_1<0.001	1.79±0.04 p_1<0,002	1.01±0.05 p_1<0.001 p_2<0.001 p_3<0.001

Notes: p_1 – statistical significance of the differences compared to the intact animals;
p_2 – statistical significance of the differences compared to the animals with experimental passive tobacco smoking;
p_3 – statistical significance of the differences compared to the rats affected by monosodium glutamate.

glutamate injection – by 15.0%, in cases of prolonged administration of monosodium glutamate – by 7.6%; the intensity of changes in succinate dehydrogenase activity was lower with respect to those in the mature male rats.

In the immature male rats, passive tobacco smoking is accompanied by a decrease succinate dehydrogenase (SDG) activity in mitochondria of blood leukocytes by 42.9% ($p<0.001$) compare to the control group. Passive tobacco smoking combined with the monosodium glutamate injection is accompanied by a more significant decrease in succinate dehydrogenase activity (in 3.4 times, $p<0.001$) compare to the control group, which is 48.8% ($p<0.001$) below this indicator, subject to the isolated effect of tobacco smoke. In this case, prolonged administration of monosodium glutamate results in a less significant decrease in succinate dehydrogenase activity (by 27.1%, $p<0.001$) compared to the control rats.

In the immature male rats, the changes of succinate dehydrogenase activity exceed the indicators of the mature male rats of all experimental groups: in cases of modeled passive tobacco smoking – by 16.3 %, with the introduction of monosodium glutamate – by 9.9 %, in cases of modeled passive tobacco smoking combined with the monosodium glutamate injection – by 23.7 %.

Unidirectional changes are evidenced relation to the end-stage enzyme of mitochondrial respiratory chain – cytochrome oxidase (CO). In the mature male rats with modeled passive tobacco smoking, the activity of CO in mitochondria of blood leukocytes in the mature male rats decreased by 15.5% ($p<0.05$) compare to the control group. In the animals with modeled passive tobacco smoking combined with the monosodium glutamate it is also accompanied by a decrease in cytochrome oxidase activity (by 27.5% ($p<0.001$) compare to the control group, which does not significantly differ from this indicator, provided isolated effect of tobacco smoke. At the same time, prolonged administration of monosodium glutamate leads to a decrease in the activity of this enzyme (by 10.5 % ($p<0.05$) compared with the control rats.

In the mature female-rats, passive tobacco smoking is accompanied by a decrease in CO activity in mitochondria of blood leukocytes by 24.1 % ($p <0.001$) compare to the control group. In the animals with modeled passive tobacco smoking combined with the monosodium glutamate it is accompanied by an even greater decrease in CO activity (by 37.7%, $p<0.001$) compare to the control group, which is 17.8% ($p<0.05$) below this indicator, provided isolated effect of tobacco smoke. In this case, prolonged administration of monosodium glutamate do not lead to a significant reduction in CO activity compared with the control rats.

In the sex aspect, in the mature male rats with modeled passive tobacco smoking, the changes of cytochrome oxidase activity exceed by 8.6%, in cases of passive tobacco smoking combined with the monosodium glutamate – by 10.2%.

In the immature male rats, passive tobacco smoking is accompanied by a decrease in the activity of CO in mitochondria of blood leukocytes by 32.0% ($p<0.001$) compare to the control group. In the animals with modeled passive tobacco smoking combined with the monosodium glutamate it is accompanied by a more significant decrease in the activity of CO (by 53.9%, $p<0.001$) compare to the control group, which is 32.2% ($p<0.001$) below this indicator, provided isolated effect of tobacco smoke. In this case, prolonged administration of monosodium glutamate results in a significant decrease in CO activity (by 18.3% ($p<0.002$) compared with the control rats.

In the age aspect, in immature male-rats, the intensity of changes in CO activity was higher than those of the mature male-rats of all experimental groups: with passive tobacco smoking – by 16.5%, with monosodium glutamate administration – by 7.8%, in group passive tobacco smoking combined with the monosodium glutamate – by 26.4%.

Our studies have showed that the percentage of neutrophils with ROS overproduction in the mature male-rats under passive tobacco smoking significantly increases by 2.2 times ($p<0.001$) vs the control group (Table 2). Passive tobacco smoking combined with the monosodium glutamate administration is accompanied by an even greater increase in the percentage of neutrophils with ROS overproduction (by 3.1 times, $p<0.001$) vs. the control group. The prolonged administration of monosodium glutamate results in a less significant decrease in the percentage of neutrophils with ROS overproduction (by 40.3%, $p<0.001$) compared to the control rats.

In the mature female rats, passive tobacco smoking is accompanied with the increase in the percentage of neutrophils with ROS overproduction by 3.0 times ($p<0.001$) compared to the control group. Passive tobacco smoking

Table 2. Influence of passive tobacco smoking and monosodium glutamate on the percentage of neutrophils with ROS overproduction of rats (M±m, n=8)

Indicator	Groups of experimental animals			
	Intact	Passive tobacco smoking	Monosodium glutamate	Passive tobacco smoking+ monosodium glutamate
Mature male-rats				
ROS^+-cells, %	17.98±0.86	39.44±2.56 $p_1<0.001$	25.23±1.19 $p_1<0.001$	56.39±2.82 $p_1<0.001$ $p_2<0.01$ $p_3<0.001$
Mature female-rats				
ROS^+-cells, %	14.95±0.98	45.21±1.70 $p_1<0.001$	18.71±0.78 $p_1<0.02$	60.95±3.07 $p_1<0.001$ $p_2<0.02$ $p_3<0.001$
Immature male-rats				
ROS^+-cells, %	12.90±0.77	36.89±1.62 $p_1<0.001$	21.85±0.87 $p_1<0.001$	51.39±2.60 $p_1<0.001$ $p_2<0.002$ $p_3<0.001$

Notes: p_1 – statistical significance of the differences compared to the intact animals;
p_2 – statistical significance of the differences compared to the animals with experimental passive tobacco smoking;
p_3 – statistical significance of the differences compared to the animals affected by monosodium glutamate.

combined with the MSG administration is accompanied by an greater increase in the percentage of neutrophils with ROS overproduction (by 3.7 times, p<0.001) compared to the control group. At the same time, prolonged administration of MSG results in an increase in the percentage of neutrophils with ROS overproduction by only 25.1% (p<0.02) compared to the control rats.

The percentage of neutrophils with ROS overproduction in the immature male-rats under passive tobacco smoking significantly increases by 2.8 times (p<0.001) vs the control group. Passive tobacco smoking combined with the MSG administration is accompanied by the greater increase in the percentage of neutrophils with ROS overproduction (by 4.0 times, p<0.001) vs. the control group. The prolonged administration of MSG results in a less significant increase in the percentage of neutrophils with ROS overproduction (by 69.4%, p<0.001) compared to the control rats.

Discussion

The most important functions of mitochondria are oxidation of intermediate carbohydrate, lipid and protein metabolites such as pyruvate, fatty acids, acetate, and use of energy released upon decomposition of these compounds for the biosynthesis of ATP. Mitochondrial dysfunctions, associated with oxidative phosphorylation processes, structural integrity of mitochondria and information identity of their genetic apparatus, intensify in cases of oxidative stress, in diseases caused by metabolic disorders, and carcinogenesis [9].

Mitochondrial respiratory chain is the main intracellular source for generation of reactive oxygen species (ROS), and the activity of succinate dehydrogenase as a component of the 2nd complex of the respiratory chain largely determines the rate of use of oxygen and synthesis of ATP in mitochondria [10]. Both succinate dehydrogenase and cytochrome oxidase determine the functioning of the chain of transformations of energy substrates [11].

Consequently, the intensity of energy processes in cases of passive tobacco smoking combined with the monosodium glutamate is significantly reduced in the animals of all experimental groups, which ultimately leads to 'energy starvation'. Decrease in cellular respiration and cellular energy abnormalities may

be caused by endogenic intoxication and oxidative stress.

Mechanisms of tobacco-induced oxidative stress are primarily due to the fact that tobacco smoke is a substance that is directly a source of ROS as superoxide anion radical, hydrogen peroxide, and hydroxyl radical. In general, according to Yanbaeva D.G. and coauthors, tobacco smoke contains 10^{17} molecules of oxidants per one breath [12]. In addition, activation of inflammatory cells induced by tobacco smoke promotes the production of oxidants in tissues.

Many components of tobacco smoke can accumulate in mitochondria and affect the function of the respiratory chain, thereby affecting the cellular generation of *adenosine triphosphate* (ATP). In particular, carbon monoxide can interact with the components of the mitochondrial respiratory chain and suppress cytochrome oxidase [13].

Lykhatskyi P. H. et al. in the experiment on rats of different ages exposed with tobacco smoke for 45 days had established the inhibition of mitochondrial enzymes activity, indicating violations of bioenergetics processes in the body [14]. The most pronounced changes they had observed were in immature and senile rats.

In cases of administration of MSG, the mitochondrial respiratory chain is the main source of ROS. In addition, the increase in extracellular level of glutamate increases production of hydroxyl radicals. Studies by Sharma A. proved increased activity of α-Ketoglutarate dehydrogenase (α-KGDH) in cases of the use of MSG, which could activate the oxygen and form anion superoxide and hydrogen peroxide [15].

Most researches interweave oxidative stress and tissue damage through glutamate receptors. Glutamate receptors include three families of ionotropic receptors (NMDA – N-methyl-D-aspartate, AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate) and three groups of metabotropic receptors (mGluR) [5]. Activation of mGluR produces oscillatory increases in intracellular Ca^{2+}. Increased Ca^{2+} influx leads to excessive uptake of Ca^{2+} into mitochondria, which can increase generation of ROS. Generation of ROS by Ca^{2+}-loaded polarized mitochondria depletes the antioxidant potential of a cell, which causes final disruption of cytoplasmic homeostasis of calcium that can trigger numerous cellular reactions, including activation of nitric oxide synthase, protein kinase C and inhibition of mitochondria enzymes activity [16].

Studies by S. Wu have proved that oxidative stress can even lead to fragmentation of mitochondria [17]. A study by S. Kumari et al. [18] showed that the glutamate induced cytotoxicity was associated with mitochondrial hyperpolarization, increased ROS production and enhanced oxygen consumption, glutamate-caused mitochondrial dynamic imbalance and reduced the number of cells with fragmented mitochondria, up to the splitting and activated autophagy.

Analyzing the correlation interactions between generation of ROS by blood neutrophils and their bioenergetics processes, without taking sex and age features into account, in the experimental groups of rats a negative moderate correlation ($r = -0.60$; $p<0.05$) between the generation of ROS and SDG activity and a similar interaction ($r = -0.57$; $p<0.001$) between the generation of ROS and CO activity, provided isolated effect of tobacco smoke, has been established.

Considering the division of rats by age and sex, in the mature male rats a significant negative correlation ($r = -0.85$; $p<0.05$) between the ROS generation and succinate dehydrogenase activity and a similar interaction ($r=-0.85$; $p<0.05$) between the generation of ROS and cytochrome oxidase activity, provided isolated effect of tobacco smoke. In case of passive tobacco smoking combined with the monosodium glutamate, installed an significant negative correlation ($r=-0.88$; $p<0.05$) between the generation of ROS and cytochrome oxidase activity has been evidenced.

In the mature female rats with modeled passive tobacco smoking, a significant negative correlation ($r=-0.72$; $p<0.05$) between the ROS generation and activity of cytochrome oxidase has been established.

In the immature male rats, a significant negative correlation between the generation of ROS and cytochrome oxidase activity has been established, provided isolated effect of tobacco smoke ($r=-0.78$; $p<0.05$) as well as in cases of passive tobacco smoking combined with the monosodium glutamate ($r=-0.72$; $p<0.05$).

Using ANOVA test, the influence of sex and age on succinate dehydrogenase and cytochrome oxidase activity of neutrophils mitochondria in the rats of the experimental groups has been determined. In cases of passive tobacco smoking combined with the monosodium

glutamate, an age differences in the activity of mitochondrial enzymes ($p<0.05$) have been proved.

Conclusions

Passive tobacco smoking is accompanied by an significant inhibition of bioenergetics processes in the mitochondria of circulating neutrophils in rats. The combination of passive tobacco smoking with the monosodium glutamate administration is accompanied by more pronounced changes.

In the sex aspect, the bioenergetics processes under the condition of passive tobacco smoking combined with the monosodium glutamate administration are more declining in mature female-rats, and with the age-old comparison of the changes in the activity of succinate dehydrogenase and cytochrome oxidase, was established their more intense reduction in immature male-rats.

Thus, the findings suggest that low dose intake of monosodium glutamate enhances the ability of tobacco smoke to disrupt the cell's bioenergetics processes by affecting the respiratory chain function and generation of ATP. Therefore, it is advisable to investigate the established toxic doses of monosodium glutamate, as well as to study the molecular mechanisms of 'safe' (allowed) doses of monosodium glutamate effect on a living organism.

СТАТЕВІ ТА ВІКОВІ АСПЕКТИ БІОЕНЕРГЕТИЧНИХ ПРОЦЕСІВ ПРИ ПАСИВНОМУ ТЮТЮНОПАЛІННІ ТА ЗАСТОСУВАННІ ГЛУТАМАТУ НАТРІЮ В ЕКСПЕРИМЕНТІ

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

Вступ. *Активне та пасивне паління щороку є причиною понад 5 мільйонів смертей. Водночас технічний прогрес у харчовій та переробній областях сприяє використанню харчових добавок, які не завжди безпечні для здоров'я людини, наприклад, глутамату натрію.*

Мета дослідження – *визначити зміни активності мітохондріальних ферментів у щурів при пасивному палінні у поєднанні з тривалим введенням глутамату натрію в статевому та віковому аспектах.*

Методи дослідження. *Оцінку біоенергетичних процесів проводили за допомогою визначення активності сукцинатдегідрогенази та цитохромоксидази в мітохондріях циркулюючих нейтрофілів.*

Результати. *Пасивне паління у поєднанні з введенням глутамату натрію у зрілих щурів-самців супроводжується значним гальмуванням біоенергетичних процесів, про що свідчить зниження активності сукцинатдегідрогенази на 47,1 % ($p<0,001$) порівняно з інтактними тваринами, що на 27,9 % ($p<0,001$) нижче цього показника при ізольованому ефекті тютюнового диму; і зниженні активності цитохромоксидази на 27,5 % ($p<0,001$) порівняно з контрольною групою.*

Висновки. *Таким чином, отримані дані свідчать про те, що глутамат натрію підвищує здатність тютюнового диму порушувати процеси біоенергетики клітини, впливаючи на функцію дихального ланцюга і генерацію АТФ. Тому доцільно досліджувати ефекти встановлених токсичних доз Е621, а також вивчати молекулярні механізми впливу його "безпечних" (дозволених) рівнів на організм.*

КЛЮЧОВІ СЛОВА: **пасивне тютюнопаління; глутамат натрію; цитохромоксидаза; сукцинатдегідрогеназа.**

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