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IMPROVEMENT IN THE APPROACHES TO THE TREATMENT OF EMIGRANTS AND RE-EMIGRANTS WITH DEPRESSIVE DISORDERS

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Background. *Social and clinical relevance of emigration and re-emigration is determined by a significant amount of Ukrainians that emigrate and re-emigrate. Clinical features of depressive disorders have not been studied;as well as risk factors and antiriscs of various forms of depression have not been established yet. Development of comprehensive approaches to the treatment and rehabilitation of these patients is an effective way of resolving of this problem and is of significant scientific, practical, medical and social importance.*

Objective. *The development of measures for treatment of depressive disorders in emigrants and re-emigrants is based on investigation and analysis of clinical-psychopathological phenomenology of depression.*

Methods. *We examined 196 emigrants, 191 re-emigrants and 198 not emmigrants with final clinical diagnosis of depressive disorder according to ICD-10 criteria. Examination was conducted by means of depression rate according to M. Hamilton, the Montgomery-Asberg and Symptom Checklist-90-Revised and the scale of life quality by WHO QOL-26. Statistical analysis of the results was performed using nonparametric methods: Mann-Whitney test, Wilcoxon criterion of signs.*

Results. *It was found that the maximum content of organic acids is accumulated in grass Hyssopus officinalis L. (Lamiaceae), and the minimal is in the leaves of Chrysánthemum xhortorum L. variety Apro (Asteraceae). The dominance of aliphatic acids was determined by means of gas chromatography. Benzoic is predominant among aromatic acids.*

Conclusions. *The suggested therapy schemes proved higher effectiveness compared with the traditional one, and also allowed to improve the life quality of emigrants and re-emigrants.*

KEY WORDS: **depressive disorders; emigrants; re-emigrants; clinical-psychopathological features.**

Introduction

Emigration and re-emigration is one of the most important and actual public and social matter. The number of Ukrainian emmigrants is estimated up to 6.5–7 million people, what is about 15% of the population, and tends to increase [7]. Re-emigration (coming back of emigrants) is independent medical and social problem; its amounts are comparable with migration. Migration is a traumatic factor, provoking manifestation and exacerbation of endogenous mental disorders [1]. In the structure of personality changes in emigrants, emotional instability, anxiety, social introversion, behavioral conformity, suspicion, poor integration of personality traits as emotivity and frustration, low level of frustrational tolerance are detected. Among mental disorders associated with emigration, psyhodisadaptative states, posttraumatic stress disorder, neurasthenia, prolonged depressive reaction and moderate depressive episode are the most common.

However, despite the significance of the problem of emigration and re-emigration important issues related to the course of depressive disorders in emigrants and re-emigrants are have not been studied, treatment-rehabilitation programs have not been developed and implemented for emigrants and re-emigrants with depressive disorders.

The aim of the study is the development and improvement of measures for treatment of depressive disorders in emigrants and re-emigrants due to investigation and analysis of clinical-psychopathological phenomenology of depression.

Methods

We examined 196 persons who for at least one last year lived outside Ukraine and planned to return abroad soon (emigrants); and 191 persons who at least a year lived outside Ukrai-

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ne and over the last year have returned for permanent residence in Ukraine (re-emigrants). Also we included 198 persons which were permanently living in the Ukraine, and never traveled abroad for long stay (not emmigrants). All of the patients were treated in the Ternopil Regional Municipal Clinical Psychoneurological Hospital for the period from 2010–2014 years, with established final clinical diagnosis of depressive disorder according to ICD-10 criteria. Psychogenic depressive disorder (ICD-10 codes F43.21 and F43.22) was established in 69 of not emigrants, 68 of emigrants and 67 re-emigrants, endogenous (ICD-10 codes F31.3, F31.4, F32.1, F32. 2, F33.1 and F33.2) – in 65, 66 and 63 persons accordingly, organic (ICD-10 code F06.3) – in 64, 62 and 61 people accordingly. Examination was conducted by means of depression rate according to M. Hamilton [8], the Montgomery-Asberg [9] and questionnaire of psychopathological symptoms severity Symptom Checklist-90-Revised [10] and the scale of life quality by WHO QOL-26. Statistical analysis of the results was performed by nonparametric methods: Mann-Whitney test, Wilcoxon a criterion of signs.

Results

The determined in our study clinical-psychopathological and patopersonal changes in emigrants and re-emigrants prove the necessity of improvement of psychiatric help for this group of patients. Today in Ukraine the absence of targeted treatment and rehabilitation programs for re-emigrants and emigrants determines the necessity of development of funda_ mental principles, aims and approaches to the treatment of depressive disorders in these patients.

Considering the clinical-psychopathological and patopersonological features evidenced during the examination of the emigrants and re-emigrants with depressive disorders of different genesis, we recommended the following schemes of medicational therapy and psychotherapy.

The suggested scheme of treatment and rehabilitation due to the principles of an integrated, individual and differentiated approach, ensure the staging and continuity of treatment and rehabilitation. Its objectives defined as more rapid elimination of depressive disorders (depression, anxiety-depressive, asthenic-depressive, apatho-depressive syndromes), maximal recovery and social functioning of patients, prevention of recurrence of depressive disorders, providing early socialization and social adaptation considering the features found in the emigrants and re-emigrants, engaging families and public organizations. Clinical features of depressive disorders (depressed mood, anhedonia, fatigue, anxiety, agitation, etc.), social desadaptation and disorders of microsocial interaction, abnormal behavioral patterns associated with the presence of depressive disorders, as well as factors of emigration and re-emigration were defined as therapy goals.

The suggested scheme was realized in four stages that consisted of succession and continuity of treatment and diagnostic measures. The first stage – diagnostical – included clinical psychopathological assessment of disorders that were evidenced in the patient's psychoemotional sphere, analysis of anamnesis, clinical symptoms, dynamics and prognosis of the disease, the relationship of clinical and psychosocial factors. The second stage – complex treatment – included a compound of biological therapy and psychotherapy aimed to relieve depressive disorder, normalization of emotional state, social adaptation and readaptation. Antidepressant therapy at this stage included prescription of agomelatine in a daily dose of 25 mg for the emigrants suffering from psychogenic depressive disorders, for the patients with endogenous depressive disorders – quetiapin in a daily dose of 200 mg, for the emigrants with depressive disorders of organic genesis – fluoxetinein a daily dose of 20 mg, for the re-emigrants with psychogenic depressive disorders – mirtazapine in a daily dose of 30 mg, for the re-emigrants with endogenous depressive disorders – combination of fluoxetine and risperidone in a daily dose of 20 mg and 2 mg accordingly, for the re-emigrants with depressive disorders of organic genesis – fluoxetine in daily dose of 20 mg. Differentiated psychotherapeutic correction for the emigrants included rational use of psychotherapy, cognitive behavioral therapy and group psychotherapy, and for the emigrants suffering from psychogenic depressive disorder – also psyhoeducational therapy. Psychotherapeutic interventions for the re-emigrants consisted of rational, family, cognitive-behavioral therapy; the focus was on working with the automatic thoughts and dysfunctional beliefs. The criterion of treatment efficacy was stable (at least two weeks) normalization of emotional state, disappearance of clinical signs of depressive disorder, working out adequate emotional response to real-life circumstances, including emigration (re-emi-

gration), disactualization of destructive and formation of constructive patterns of behavior. The third stage – rehabilitation – aimed at forming stable adequate emotional-behavioral pattern, maximum adaptation and readaptation of a patient, preventing of depressive disorder relapse, while medicamental therapy was similar to that used during the treatment with the appropriate dosage correction due to the actual condition of the patient. Differentiated psychotherapeutic work included the use of cognitive-behavioral therapy for the emigrants, and a combination of cognitive-behavioral therapy, family therapy and autogenous training for the re-emigrants. At this stage, also the measures for rehabilitation and social reintegration of the patients were also useful. The fourth stage – preventive – aimed at maintaining of normal emotional state, effective stress resistance and prevention of depressive disorder recurrence. Medical therapy included treatment of primary disease in cases of depressive disorders of organic genesis and seasonal prevention of depressions of endogenous character and psychotherapy – techniques of self-regulation, and measures for social adaptation.

Comparison analysis of effectiveness was conducted in three main directions: 1. Dynamics of mental state (recovery, significant improvement of mental state, improvement of mental state, lack of dynamics of mental state, worsening of mental state). 2. Dynamics of pathological manifestations severity according to psychometric scales. 3. Dynamics of life quality rate during the treatment. Reassessment performed 6 months after the beginning of the treatment.

To evaluate the effictiveness of the suggested treatment, regimens were divided into 6 major groups due to the emigrants and re-emigrants suffering from psychogenic, endogenous and organic depressive disorders, receiving the suggested treatment and 6 similar in all clinical and social characteristics comparison groups. Comparison of the results was conducted in pairs of each clinical group due to the clinical variant and genesis of depressive disorder. Comparing analyzes of effectiveness was conducted in three main directions: dynamics of mental state, dynamics of pathological manifestations severity according to psychometric rate and dynamics of life quality during treatment.

Assessment of mental state dynamics as a result of the conducted therapy proved significantly higher efficiency of the offered scheme of treatment compared to the traditional one (Fig. 1).

In the main group higher quality of treatment was achieved: in the group of the emigrants suffering from psychogenic depressive disorders in 81.2% of the patients clinical recovery, in 9.4% – a significant improvement, in 6.3% – improvement, no dynamics was detected in one patient (3.1%), cases of reverse dynamics of depressive disorders under the influence of treatment were not found, while in the control group of patients clinical recovery was achieved in 42.4% of cases, significant improvement – in 33.3%, improvement – in 15.2%, no changes – in 6.1%, and in one case (3.0%) inverse dynamics ($p<0.05$) was evidenced. In the emigrants with endogenous depressive disorder, who received the suggested scheme of treatment, clinical recovery was achieved in 59.3% of cases, significant improvement – in 21.9%, improvement – in 12.5%, lack of dynamics – in 6.3%, in the group of patients receiving traditional therapy clinical recovery was achieved in 24.2%, a significant improvement and improvement – in 27.3%, no dynamics was detected in 18.2%, worsening of state – in one case (3.0%) ($p<0.05$). In the group of emmigrants with depressive disorders of organic genesis, clinical recovery was achieved in 36.6%, a signi-

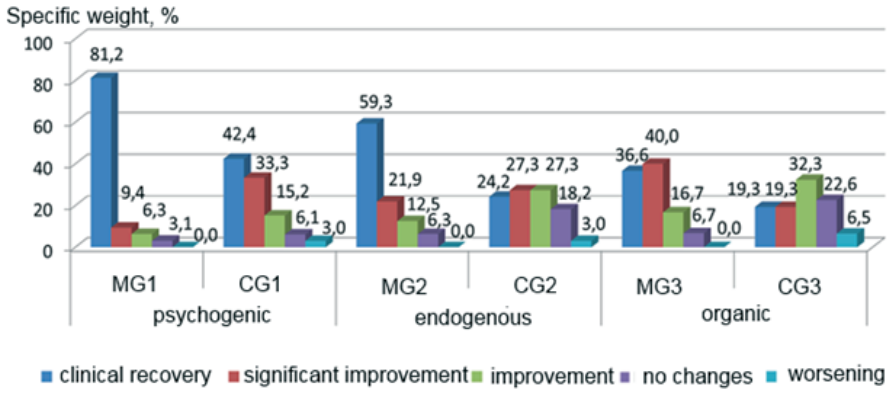

Fig. 1. Comparison of the results of traditional and suggested treatment schemes for the emmigrants, patients with depressive disorders.

ficant improvement – in 40.0%, improvement – in 16.7%, lack of dynamics – in 6.7%, in the comparison group clinical recovery was found in 19.3%, a significant improvement – in 19.3%, improvement – in 32.3%, no explicit dynamics – in 22.6%, worsening of state – in 6.5% ($p<0.05$).

In the re-emigrants with depressive disorders higher effectiveness of the suggested scheme was also proved (Fig. 2).

In the re-emigrants suffering from psychogenic depressive disorder treated by the suggested scheme, clinical recovery was achieved in 71.0% of cases, significant improvement – in 16.1%, improvement – in 9.7%, the lack of dynamics occurred in one case (3.2%), the inverse dynamics was not detected, and there-emigrants with psychogenic depressive disorders who received conventional therapy achieved clinical recovery in 32.2%, a significant improvement – in 35.5%, improvement – in 22.6%, no dynamics was detected in 6.5%, inverse dynamics – in 3.2% of the patients ($p<0.05$). In the re-emigrants with endogenous depressive disorders, treated by traditional therapy, clinical recovery was achieved in 51.5% of cases, significant improvement – in 22.6%, improvement – in 19.4%, no dynamics was detected in 6.5% of the patients. After the treatment by the suggested clinical therapy, recovery and significant improvement occurred in 19.3%, improvement – in 42.0%, lack of dynamics – in 12.9%, inverse dynamics – in 6.5% of the patients ($p<0.05$). In the re-emigrants with depressive disorders of organic genesis, the suggested scheme has lead to clinical recovery in 33.3%, a significant improvement – in 36.7%, improvement – in 20.0%, no dynamics was detected in 10.0%, and in the comparison group of clinical recovery occurred in 13.3%, a significant improvement – 20.0%, improvement – in 40.0%, lack of dynamics – in 16.7%, state worsening – in 10.0% of the patients ($p<0.05$).

Discussion

Due to the the intended aim and objectives of our study the analisis of the received results made it possible to justify the treatment and rehabilitation programs for the emigrant and re-emigrant patients with depressive disorders. Considering the features of depressive disorders in these groups of patients and lack of information on the developed approaches to treatment and rehabilitation, we suggested new therapeutic schemes. Therefore, we invite you to the discussion of scientific research that proved positive results.

Analysis of quantitative indices by scale of depression according to M. Hamilton also confirmed significantly higher efficiency of the suggested scheme for both the emigrant and re-emigrant patients with psychogenic, endogenous and organic depressive disorders.

So, the suggested scheme of therapy for the emigrants suffering from psychogenic depressive disorder was significantly more effective against depressed mood, guilt, suicidal intentions, early, middle and late insomnia, working capacity and activity, retardation, agitation, mental anxiety, somatic symptoms, genital symptoms and hypochondria. The suggested scheme also promoted to significant decrease of the overall rate of depression and indicators of adynamic, agitated depression, depression with fear and undifferentiated depression.

The suggested scheme of treatment enabled achievement of significantly improved indexes of main subrates of M. Hamilton depression scale in the emigrants with endogenous depressive disorders. Significant differences were detected by comparison of the indexes after reasonable treatment of depressed mood, guilt, early, middle and late insomnia, working capacity and activity, retardation, agitation, mental anxiety, somatic symptoms and hypochondria, as well as general indicators of de-

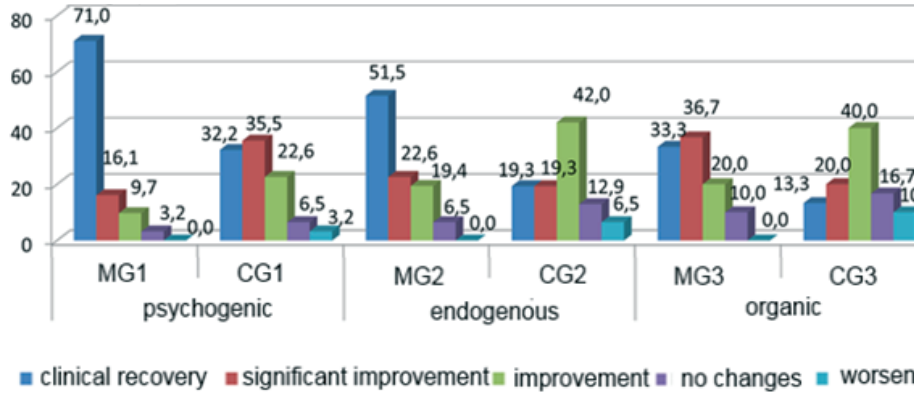

Fig. 2. Comparison of the results of traditional and suggested treatment schemes for the re-emigrants with depressive disorders.

pression, indexes of adynamic, agitated depression, depression with fear and undifferentiated depression.

For the emigrants suffering from depressive disorders of organic genesis, the suggested scheme of treatment was significantly more effective against depressed mood, guilt, suicidal intentions, early, middle and late insomnia, working capacity and activity, retardation, agitation, mental anxiety, somatic symptoms, genital symptoms and hypochondria, as well as general indicators of depression, indexes of adynamic, agitated depression, depression with fear and undifferentiated depression.

The suggested scheme of treatment also allowed decreasing anxiety in the re-emigrants and the emigrants with depressive disorders of different genesis.

For the emigrants suffering from psychogenic depressive disorders, the suggested scheme was significantly more effective against anxious mood, tension, anxiety, insomnia, depressed mood, muscle somatic symptoms, somatic sensory symptoms, cardiovascular symptoms, respiratory symptoms, hastrointestional symptoms, autonomic symptoms, as well as general severity of psychic and somatic anxiety.

For the emigrants with endogenous depressive disorder, the suggested scheme was significantly more effective against anxious mood, tension, insomnia, depressed mood, muscle somatic symptoms, somatic sensory symptoms, cardiovascular symptoms, respiratory symptoms, vegetative symptoms, as well as general severity of psychic and somatic anxiety.

For the emigrants suffering from depressive disorders of organic nature, the suggested scheme was significantly more effective against anxious mood, tension, anxiety, insomnia, depressed mood, muscle somatic symptoms, cardiovascular symptoms, respiratory symptoms, vegetative symptoms, as well as general severity of psychic and somatic anxiety.

In the re-emigrants the positive influence of the suggested scheme was evidenced. Thus, for the re-emigrants suffering from psychogenic depressive disorder, the suggested scheme was more effective against anxious mood, tension, anxiety, insomnia, cognitive disorders, depressed mood, muscle somatic symptoms, somatic sensory symptoms, cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, vegetative symptoms, as well as general severity of psychic and somatic anxiety.

The suggested scheme of treatment for the re-emigrants with endogenous depressive disorder, was more effective against anxious mood, tension, anxiety, insomnia, cognitive disorders, depressed mood, muscle somatic symptoms, somatic sensory symptoms, cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, vegetative symptoms, as well as the general severity of psychic and somatic anxiety.

For the re-emigrants with depressive disorders of organic genesis, the suffested scheme significantly improved the rate of anxious mood, anxiety, insomnia, muscle somatic symptoms, somatic sensory symptoms, cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms and severity of psychic and somatic anxiety.

The influence of the suggested therapy on the severity of psychopathological symptoms was also more effective compared to the traditional one.

For the emigrants suffering from psychogenic depressive disorders, the suggested scheme proved significantly better results in the rate of somatization, obsessive-compulsive disorders, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety and general indicator (index GSI), the index of symptoms detecting (PSI) and index of distress severity (PDSI).

For the emigrants suffering from depressive disorders of organic genesis, the suggested scheme was proved effectiveness in the rate of of somatization, obsessive-compulsive disorders, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety and general indicator (index GSI), the index of symptoms detecting (PSI) and index of distress severity (PDSI).

In the patients with depressive disorders of different genesis significantly better rates of psychopathological symptoms severity influenced by the suggested scheme of therapy were achieved.

Thus, the re-emigrants suffering from psychogenic depressive disorders, treated by the suggested scheme achieved more pronounced decrease in the rates of somatization, obsessive-compulsive disorders, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety and general indicator (index GSI), the index of symptoms detecting (PSI) and index of distress severity (PDSI).

For the re-emigrants with endogenous depressive disorders, the suggested therapy scheme promoted to more effective decrease in the rates of somatization, obsessive-compul-

sive disorders, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety and general indicator (index GSI), the index of symptoms detecting (PSI) and index of distress severity (PDSI).

For the re-emigrants with depressive disorders of organic genesis, the suggested treatment scheme was more effective for decrease in rates of somatization, obsessive-compulsive disorders, interpersonal sensitivity, anxiety, hostility, phobic anxiety and general indicator (index GSI), the index of symptoms detecting (PSI) and index of distress severity (PDSI).

Significant improvement in quality of life during the treatment is an important factor that positively assesses the perspectives of implementation of the suggested treatment regimens.

Thus, in the group of emigrants suffering from psychogenic depressive disorders, the suggested scheme promoted to achievement of significant improvement of physical health and psychological QOL.

For the emigrants with endogenous depressive disorders, the use of the suggested scheme of therapy promoted to improvement of QOL in physical health, psychological QOL and environment.

For the emigrants suffering from depressive disorders of organic genesis, the suggested scheme was more effective in improvement of QOL in physical health and psychological QOL.

The improvement of QOL during the treatment by the suggested scheme was also achieved in the re-emigrants. Thus, in the re-emigrants with psychogenic depressive disorders, significantly higher QOL of physical health and psychological QOL were defined, in the re-emigrants with endogenous depressive disorders – of QOL in physical health, psychological QOL and environment, in the re-emigrants with depressive disorders of organic origins – of QOL in physical health, psychological QOL and environment.

Thus, the comparative analysis of the suggested treatment schemes for the emigrants and re-emigrants suffering from psychogenic, endogenous and organic depressive disorder proved significantly higher effectiveness comparing with traditional one.

Conclusions

The analysis of the influence of the suggeste therapy schemes on the severity of psychopathological symptoms also proved higher effectiveness compared with the traditional one; it was evidenced by significant decrease in the rate of somatization, obsessive-compulsive disorders, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, as well as general index, the index of symptoms detection and the index of distress severity.

The suggested therapy scheme of also promoted to improvement of life quality of the emigrants and re-emigrants with depressive disorders, significant increase in the rates of their physical health, psychological quality of life and environment compared to the comparison group.

The results allow recommending the developed treatment scheme for implementation in the complex treatment of emigrants and re-emigrants suffering from psychogenic, endogenous and organic depressive disorders.

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SCREENING FOR DYSLIPIDEMIA AND EXPEDIENCE OF STATIN THERAPY FOR THE CITIZENS OF TRANSCARPATHIA VALLEY REGIONS WITH OVERWEIGHT AND OBESITY

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Background. *The lipid profiles of patients with overweight, obesity and healthy individuals, the citizens of Transcarpathia valley regions were analysed.*

Objective. *The study was aimed at evaluation of dyslipidaemia frequency in patients with overweight and obesity, determination of expedience of statins prescription.*

Methods. *All patients were divided into 2 groups: group 1 – patients with overweight; group 2 – patients with obesity of I and II degree. Estimation of lipid profile parameters was conducted by means of spectrophotometric device SIEMENS Dimension RxL Max. Statistical analysis of the data was conducted using Microsoft Excel 2007.*

Results. *The patients with obesity had higher level of total cholesterol (6.03±0.53 mmol/l), lower HDL-C (1.15±0.07 mmol/l) and higher level of LDL-C (4.19±0.46 mmol/l) compare with overweight patients. In 46% of patients with overweight, dyslipidaemia was evidenced and required correction, 27% of them had high CVR and needed statin therapy, 19% of people with obesity had moderate CVR and didn't need statins. 77% of obese patients needed lipid correction, 54% of them with very high and averagely high level of CVR required statin therapy; 23% of people with obesity had moderate CVR and did not need statins.*

Conclusions. *In the studied overweight and obese patients, atherogenic dyslipidaemia was established in 46% and 77% of cases respectively. Correction of dyslipidaemia with statin was compulsory for 27% of patients with overweight and for 54% with obesity.*

KEY WORDS: **body mass index; overweight; obesity; lipid profile; statins.**

Introduction

In 2000, the WHO first suggested using the term 'epidemic' to distinguish the situation with obesity prevailing in the world; it was recognized that the number of people with overweight and obesity is progressively increasing.

Over the last 35 years the number of obese patients has increased twice, and over the last 10 years it increased by 75%. According to WHO data in 2003 there were 1.7 billion of the adult population suffering from overweight (BMI≥25 kg/m^2), the statistics in 2014 announced a new data: there were more than 1.9 billion people with overweight and obesity [1].

In 2016 the results of a meta-analysis on the dynamics of BMI in adults of over 18 years old between 1975 and 2014 were published. 1698 data on population studies was analysed, which included 19.2 million people from 186 countries. The increase of average BMI from 21.7±0.4 kg/m^2 to 24.2±0.2 kg/m^2 for men and from 22.1±0.4 kg/m^2 to 24.4±0.2 kg/m^2 for women was proved [2].

The negative effect of obesity on population is established: this pathology correlates with increased morbidity, mortality, quality of life, and is a significant socioeconomic burden for the world [3]. Several studies prove that obesity increases the cost of medical care and worsens the quality of life [4].

To reduce mortality from cardiovascular diseases in people aged under 65, the Council of Europe in 2002 declared a Plan "Heart of Europe", according to which it is necessary to reduce cholesterol level in the population by less than 5.0 mmol/l, lower blood pressure in the population to a level of <140/90 mm Hg., reduce the number of smokers by 1% per 1 year. Thus, by 2020 the mortality rate should de-

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crease by 40% (2.4% per year). To achieve this goal, primary care physicians must reorient to early detection of lipid metabolism disorders, their adequate correction and prevention of atherosclerosis associated with cardiovascular diseases and their complications [5].

In most patients with obesity such lipid disorders are observed: rising of small particles of low density lipoprotein (LDL) in blood plasma [5, 6, 7], which is often associated with increased levels of triglycerides (TG) and decreased level of high-density lipoprotein cholesterol (HDL cholesterol) [5].

Algorithm development for selection of patients for statins therapy, adequate drug choice and its efficacy control, evaluation of target level of cholesterol are the topical matter for a family doctor today.

However, the questions 'how often subclinical dyslipidaemia in obese patients is revealed', and 'how reasonable the prescription of statins for these patients is' are still open, because of the absence of supply for routine lipid profile determination in the practice of family doctors in Ukraine.

The study was aimed at evaluation of dyslipidaemia frequency in patients with overweight and obesity, determination of the expedience of statins prescription.

Methods

40 individuals who attended clinics of family medicine in Uzhhorod from October to December 2015 were examined.

We analysed the following data in the examined persons: age, sex, level of total cardiovascular risk (CVR), body mass index (BMI), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), atherogenic coefficient (AC). Estimation of lipid profile parameters was conducted by means of spectrophotometric device SIEMENS Dimension RxL Max.

The study was approved by the Ethics Committee of the Transcarpathian Regional Clinical Cardiology Dispensary. The patient had to sign the informed consent to involvement in the research.

For diagnosis of the overweight or obese we used a body mass index, which was calculated as the ratio of body weight to height squared (kg/m^2). The persons, whose BMI was from 25 to 29.99 kg/m^2 composed the group of patients with overweight. The persons with BMI from 30 to 34.99 kg/m^2 were diagnosed with obesity I degree, in the persons with BMI from 35 to 39.99 kg/m^2 obesity II degree was established. The results of lipid profile were interpreted according to guidelines of the ESC and target lipid levels of patients with different CVR.

All patients were divided into 2 groups: group 1 – patients with overweight; group 2 – patients with obesity of I and II degree (hereinafter patients with obesity). The share of patients with overweight was 55% (22 people), with obesity – 45% (18 persons). Due to the terms and conditions of the research, only those patients, who did not take statins for at least one month before the date of screening, took part in the study.

Patients' data was normally distributed. Statistical analysis of the data: the T-test and Pearson rank correlation coefficient, was conducted using Microsoft Excel 2007.

Results

The average age of group 1 patients was 57.2±1.2 years old, BMI 27.06±0.42 kg/m^2. The average age of group 2patients was 59.7±1.1 years old, BMI 35.9±1.17 kg/m^2.

Patients with obesity had the following differences in lipid spectrum compared with those who were overweight: higher level of total cholesterol (6.03±0.53 mmol/l vs 4.76±0.23 mmol/l, p=0.048), lower HDL-C level (1.15±0.07 mmol/l and 1.51±0.12 mmol/l, p=0.02) and higher level of LDL-C (4.19±0.46 mmol/l and 2.89±0.21 mmol/l, p=0.026).

In the group 1, among all, who required correction of lipid spectrum, 19% of patients had moderate CVR and didn't need statin therapy because the average level of LDL-C was 2.5±0.13 mmol/l; respectively, 27% had increased CVR and average level of LDL-C was 3.4±0.21 mmol/l and they needed statin therapy.

Among the obese 77% of patients required lipid correction. 21% of group 2 patients had a very high average level of CVR because LDL-C level was 4.4±0.32 mmol/l and they needed correction of lipid profile with statins; 33% of patients had high CVR with an average level of LDL cholesterol 3.7±0.51 mmol/l and also needed statin therapy; 23% of patients with obesity had moderate CVR and did not need statins (Fig.1).

In addition to determination of significant differences between different groups, we also conducted correlation analysis of the groups of patients. In group 1 a direct average relationship was revealed between: the level of total

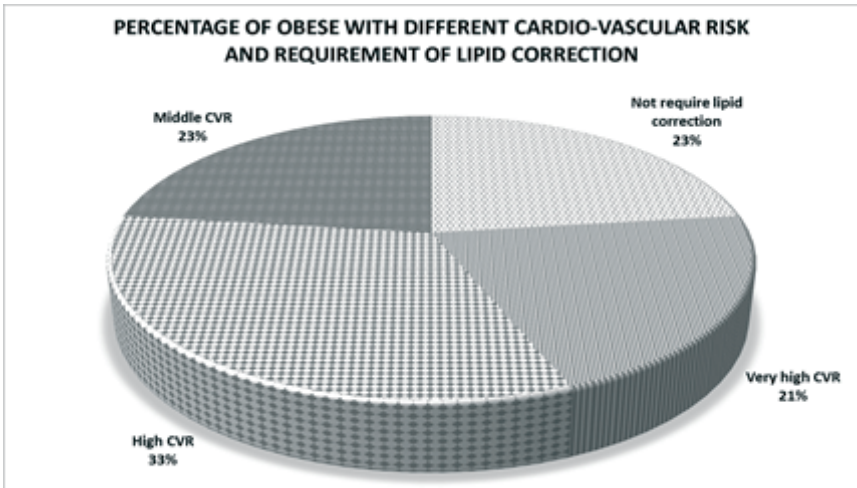

Fig. 1. Percentage of the obese with different cardio-vascular risks and lipid correction is compulsory.

cholesterol and BMI (r=0.5), BMI and LDL cholesterol (r =0.4), BMI and TG (r=0.56), BMI and atherogenic ratio (r=0.34), inverse middle relationship between age and the level of HDL cholesterol (r=-0.52). Similar correlations were found in the group 2: between BMI and total cholesterol (r=0.42), BMI and LDL cholesterol (r=0.37), BMI and TG (r=0.46).

Discussion

Thus, with increase of BMI, lipid profile got worse: the levels of total cholesterol and LDL cholesterol increased and of HDL cholesterol decreased. According to the literature [8,9, 10], in patients with overweight and obesity most often such lipid disorders are found: rising of small particles of LDL in blood plasma, which is often associated with increased level of TG and decreased level of HDL-C [6]. According to the Framingham Heart Study about 10% of patients with overweight have increased blood concentrations of cholesterol by 0.3 mmol/l. In young patients with overweight the increased level of cholesterol was twice more than in people of the same age with normal weight. According to the results of population survey conducted in the National Scientific Center "M.D. Strazhesko Institute of Cardiology" MAS of Ukraine; the occurrence of hypercholesterolemia in patients with overweight is 65%, 70% in the obese, and only 40% in people with normal weight [10].

The method of dyslipidaemia correction was chosen according to the recommendations of ESC/AHA and Association of Cardiologists of Ukraine.

46% of patients with overweight had dyslipidaemia, which required correction: lifestyle modification in 19% and/or statin therapy in 27%.

Thus, with BMI levels increase the levels of total cholesterol, LDL-C and TG significantly increase, in addition in patients of the group 1 HDL-C level decreased with age.

Conclusions

In the examined overweight and obesity patients, atherogenic dyslipidaemia was established in 46% and 77% of cases respectively.

Correction of dyslipidaemia by statin was compulsory for 27% of patients with overweight and 54% with obesity.

Regardless of gender and age, in the patients with increased BMI level, the increased levels of total cholesterol, LDL cholesterol and atherogenic factor were observed.

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LEPTIN RESISTANCE AND TYPE 2 DIABETES

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Leptin is one of adipocyte-secreted hormones. It signals to the brain and other tissues about the status of body energy reserves. Circulating leptin levels are directly proportional to the amount of the body fat. Leptin concentration increases when surfeit and decreases during fasting. Obese patients are hyperleptinemic compared with thin persons and they are tolerant to the central hypothalamic effects of leptin. The reduced sensitivity toward exogenous and endogenous leptin is commonly referred to as leptin resistance. Alterations in the signaling of the long isoform of the leptin receptor play the crucial role in leptin resistance. Surfeit may induce leptin resistance and other metabolic sequelae of obesity. Leptin insensitivity and insulin resistance play a major role in the development of type 2 diabetes. Metformin remains the preferred first-line pharmacologic agent for the treatment of type 2 diabetes. It reduces hepatic glucose production, increases glucose uptake in peripheral tissue and can lead to weight loss. Metformin decreases both insulin and leptin concentration, restores the sensitivity to these hormones. But some studies have shown poor relationship between metformin action and leptin level. And the mechanism of metformin action on leptin resistance remains unclear. Thus, these issues should be studied as well as polymorphisms in genes encoding metformin action.

KEY WORDS: **leptine resistance; type 2 diabetes; metformin.**

Leptin role in endocrine system

Adipose tissue is a complex, essential, and highly active metabolic and endocrine organ. It responds to afferent signals from traditional hormone systems and central nervous system but also expresses and secretes factors with important endocrine functions [1].

The discovery of leptin in 1994 has changed the understanding of hormonal regulation of energy homeostasis and the role of adipose tissue from that of a depot storage organ to that of an active endocrine organ producing several bioactive peptides [2].

Leptin, a 167–amino acid peptide [Fig. 1], is one of the adipocyte-secreted hormones [1].

It is also expressed in other tissues, including placenta, mammary gland, testes, ovary, endometrium, stomach, hypothalamus, pituitary, and others [3].

Leptin signals to brain and other tissues about the status of body energy reserves. Thus, that appropriate changes in food intake, energy expenditure, and nutrient partitioning can occur to maintain whole-body energy balance [4].

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Circulating leptin levels are directly proportional to the amount of the body fat [5] and fluctuate with acute changes in caloric intake [6]. Leptin concentration increases when surfeit and decreases during fasting [7].

Circulating leptin crosses blood brain barrier and mediates its action through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway (JAK–STAT3) [8].

Leptin exerts pleiotropic effects by binding and activating specific leptin receptors in the hypothalamus and other organs, has direct and indirect effects in metabolically active tissues, and regulates some neuroendocrine axes. Several isoforms of the leptin receptor, resulting from alternative splicing, convey biological activity and are involved in mediating leptin's actions in brain and peripheral organs [2].

The long leptin receptor isoform (ObRb) is expressed particularly in hypothalamus, where it regulates energy homeostasis and neuroendocrine function [9]. In ventromedial hypothalamus leptin stimulates expression of anorexigenic brain-derived neurotrophic factor [10]. In addition, leptin influences neurons that directly or indirectly regulate levels of other circulating hormones (e.g. thyroid hormone, sex steroids, and GH) [9, 11]. ObRb is also expressed in multiple peripheral tissues, including

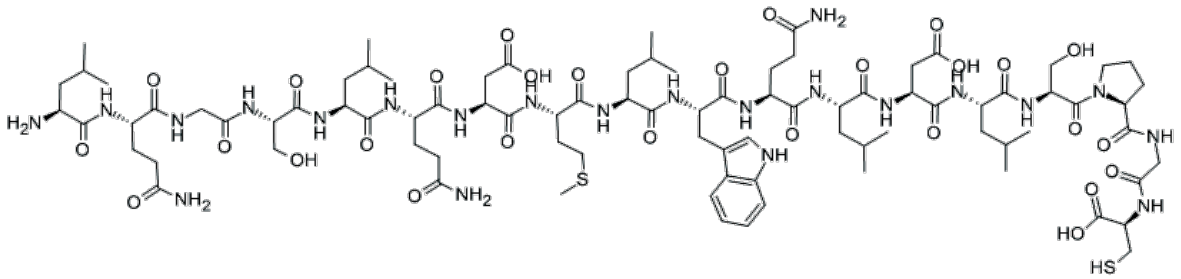


Fig. 1. Leptin structure [81].

pancreatic islets, adipose tissue, skeletal muscle, liver, and immune cells [12, 13]. In the pancreatic islets, leptin directly inhibits insulin expression and secretion [14]. In liver and white adipose tissue, leptin inhibits lipogenesis and stimulates lipolysis [12, 13]. Leptin directly promotes fatty acid oxidation in isolated adipocytes and skeletal muscle [10, 15] and decreases lipid levels in isolated livers [16].

Leptin takes part in the regulation of energy homeostasis, insulin action and lipid metabolism [1] by improving insulin sensitivity and reducing intracellular lipids [17–19].

The ability of leptin to inhibit feeding is related to activation of PI3 kinase in the hypothalamus. Blockade of PI3 kinase activity prevents the anorectic action of leptin [20].

Congenital leptin deficiency is associated with hyperphagia, impaired thermogenesis, insulin resistance, hyperlipidemia, and central hypogonadism, which can be reversed by leptin treatment [20, 21].

Women have higher leptin concentrations than men. But after menopause a significant reduction in the amount of circulating leptin occurs [22]. Such sexual differences can be explained by the difference in fat mass, body fat distribution, and sex hormones. Leptin level is also independent of body mass index. Subcutaneous fat expresses more leptin mRNA than omental (visceral) fat, and this may partially contribute to increased leptin levels in women compared with men [23].

Deficient leptin signaling results in hyperphagia and decreased energy expenditure [24]. The result is not only an increasing degree of obesity associated with increased lipid storage in muscle, liver, and other tissues but also dysfunction of several neuroendocrine axes, including the reproductive, thyroid, and adrenal axes, as well as abnormal function of the immune and autonomic system (i.e. thermoregulation, energy expenditure, and others) [17,24].

Normally leptin reduces along with insulin during fasting and mediates the suppression of thyroid hormone, growth hormone and reproductive hormones. Leptin causes stimulation of appetite and inhibition of sympathetic nerve activity [25, 26].

Chronic weight loss in females and obese patients on dietary restriction decreases leptin, resulting in suppression of reproductive hormones, disruption of menstrual cycles, and energy use by muscle [27, 28].

Leptin may control glucose homeostasis independently of its effects on adiposity [29]. It regulates glycaemia via the CNS, but it may also directly regulate the physiology of pancreatic β-cells [30, 31] and peripheral insulin-sensitive tissues [32]. It was also proved that leptin acting through LRb regulates insulin receptor substrate-1 and 2, mitogen-activated protein kinase, extracellular signal-regulated kinase, Akt, and PI3 kinase, raising the possibility of interaction between leptin and insulin [33]. Antidiabetic properties of leptin as a suppressor of glucagon are shown in some literature [34–36].

Leptin also limits accumulation of triglycerides in liver and skeletal muscle through a combination of direct activation of AMPK and indirect actions mediated through central neural pathways, thereby improving insulin sensitivity [37]. Attenuation of leptin sensitivity in brain leads to excess triglyceride accumulation in adipose tissue, as well as muscle, liver, and pancreas, resulting in impaired insulin sensitivity and secretion [38].

Although obese subjects are hyperleptinemic compared with thin persons [39], they appear either to be tolerant or resistant to the central hypothalamic effects of leptin. The reduced sensitivity toward exogenous and endogenous leptin is commonly referred to as leptin resistance [40]. However, leptin is not widely used in the clinical field because obesity is accompanied by increased serum leptin and responds poorly to the pharmacological administration of exogenous leptin, which ordinarily potently promotes fat mass loss and body weight reduction in thin subjects [41, 42].

Alterations in the signaling of the long isoform of the leptin receptor, especially in the hypothalamic arcuate nucleus, seem to play a crucial role in leptin resistance. Additional mechanisms that were proposed to induce resistance toward the effects of leptin include alterations in the transport of leptin across the blood-brain barrier. The protein tyrosine phosphatase 1B is another potential mechanism that was proposed to interfere with leptin signaling by inhibiting signaling of the long isoform of the leptin receptor [24, 43].

Leptin insensitivity and insulin resistance play a major role in the development of type 2 diabetes [44]. It can be explained by high levels of insulin and leptin as well as with hyperphagia in patients with obesity [45].

Leptin resistance as a development factor of obesity and type 2 diabetes

The WHO has declared obesity as the largest global chronic health problem in adults, and it is considered the 5th leading risk for life globally [46]. Obesity is a 'gateway' for diabetes [47].

Worldwide, 415 million people have diabetes, and this number will reach 642 million by 2040 [48]. Type 2 diabetes accounts for 90-95% of all diabetes.

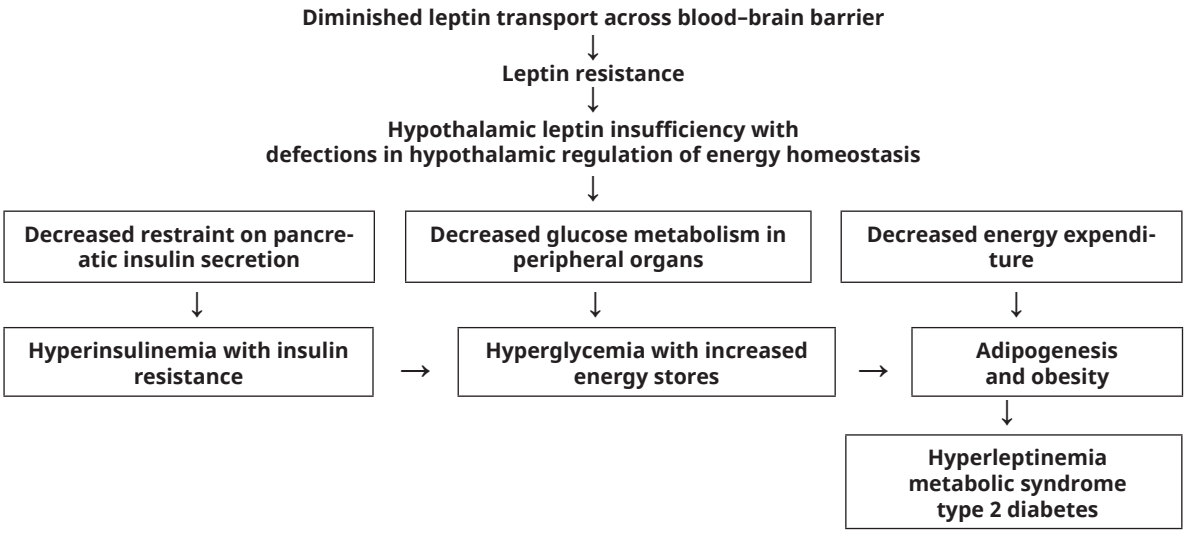
This form encompasses individuals who have relative (rather than absolute) insulin deficiency and have peripheral insulin resistance [49]. Nearly 90% of individuals with type 2 diabetes are either overweight or obese [50]. Excess weight itself causes some degree of insulin resistance. Patients with type 2 diabetes may have insulin levels that appear normal or increased; the increased blood glucose levels in these patients would be expected to result in even higher insulin values and to have their b-cell function in norm. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance [49].

Leptin resistance was introduced in an apparent analogy with that of insulin resistance to explain why hyperleptinemia associated with obesity fails to correct the defect in energy balance and feeding behaviour [51]. In obesity, the transport of leptin across the blood brain barrier is decreased and levels of suppressors of cytokine signaling-3 (SOCS-3), an inhibitor of leptin signaling is increased in hypothalamus, which leads to leptin resistance [52]. Therefore, the concept of 'leptin resistance' has been challenged recently by an alternate concept of 'hypothalamic leptin insufficiency' [53].

It is assumed that leptin resistance contributes to hyperphagia [54, 55]. It is also possible that surfeit may induce leptin resistance and other metabolic sequelae of obesity [45]. This rapid adaptation to increased energy availability may be designed to curtail the leptin system to facilitate storage of nutrients into lipid stores [54, 56]. This may be accomplished by restraining leptin biosynthesis [57–59] and/or by inducing leptin resistance [54, 56]. These mechanisms would be particularly well developed in individuals predisposed to weight gain and diabetes [54, 60]. Consistent with the 'thrifty genotype' hypothesis, this sequence of events would be tightly coupled to the onset of insulin resistance [45, 54].

Inadequate early increase in leptin secretion and biosynthesis (Table 1) in response to overeating may also play a role in the development of obesity and glucose intolerance [61].

Table 1. The scheme of metabolic disorders in the human body initiated by leptin resistance (based on the Fig. 2 by Kalra S.P., 2008)



Metformin action in patients with leptin resistance

Metformin remains the preferred first-line pharmacologic agent for the treatment of type 2 diabetes [62]. The use of metformin as a first-line therapy was supported by the findings of a large meta-analysis with selection of second-line therapies based on patient-specific considerations [63].

Metformin acts mainly by reducing hepatic glucose production via inhibition of gluconeogenesis [64, 65] and increases glucose uptake in peripheral tissue [65]. Metformin is associated with a low risk of hypoglycemia and is weight neutral or can lead to weight loss [66].

Moreover, metformin decreases leptin concentration in morbidly obese subjects [67, 68] and in normal-weight healthy men [69]. Although leptin concentration is closely related to body fat mass, the leptin-reducing effect of metformin cannot be fully explained by body weight reduction because metformin reduces leptin level even without changing body weight in normal-weight healthy men [69].

It was proved that weight loss achieved by metformin was correlated with pre-treatment plasma leptin levels. This effect of metformin was paralleled by a stimulation of the expression of the leptin receptor gene (ObRb) in the arcuate nucleus. Thus, identifying the hypothalamic ObRb as a gene was modulated after metformin treatment [70].

It has been recently reported that metformin targets the AMP-activated protein kinase (AMPK), which is also activated by leptin [71–73].

Several researches have been performed to analyse the molecular mechanism behind the effect of metformin on leptin levels. An in-vitro study reports that metformin inhibits leptin secretion by inhibiting MAPK signaling pathway in adipocytes [74].

The other studies has proved that metformin restores leptin sensitivity in obese rats with leptin resistance and metformin treatment increases cerebrospinal fluid leptin concentrations in both standard chow and high fat-fed obese rats compared with the untreated rats. The authors have also reported that metformin increases hypothalamic POMC (an anorexigenic peptide) expression by leptin treatment in high fat-fed obese rats, whereas this is not observed in untreated high fat-fed obese rats [75].

As the effect of leptin is associated with the activation of POMC, failure to activate POMC expression by leptin is an evidence of leptin resistance [76].

Distinct reduction in serum leptin level was observed in non-obese healthy individual on metformin, without any reduction in body weight [77].

But some studies have shown poor relationship between metformin action and leptin level [78, 79].

Although many researches have been conducted, the mechanism of metformin action on leptin resistance remains unclear. Moreover, similar investigations have contrary outcomes. Therefore, this issue requires further research.

Conclusions

Leptin, the adipocyte-secreted hormone, has direct and indirect effects on metabolically active tissues, and regulates several neuroendocrine axes. It takes part in the regulation of energy homeostasis, insulin action and lipid metabolism, and signals primarily on the status of the body energy reserves in fat to brain and other tissues. Circulating leptin levels are directly proportional to the amount of body fat. Leptin concentration are increased in obesity and decreased during fasting. In obesity, the transport of leptin across the blood brain barrier is diminished, which leads to leptin resistance. Leptin insensitivity and insulin resistance play a major role in the development of type 2 diabetes.

Metformin decreases both insulin and leptin concentration, restores the sensitivity to these hormones.

But the mechanism of metformin action on leptin resistance remains unclear. The leptin-reducing effect of metformin cannot be fully explained only by body weight reduction. Furthermore, some studies have proved poor relationship between metformin action and leptin level. All these aspects require an in-depth research.

Considering the topical matter of exploring metformin action due to polymorphisms in genes encoding drug receptors, transporters, and metabolizing enzymes [80], the study of the relationship between genetic variants in solute carrier transporters and changes in leptin levels under metformin therapy would be reasonable.

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THE EFFECT OF L-ARGININE ON OXIDATIVE STRESS AND MICROALBUMINURIA IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CHRONIC KIDNEY DISEASE

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Background. *Diabetic kidney disease (DKD) is one of the severest complications of diabetes. Microalbuminuria (MAU) is one of the first signals of DKD and an important pathogenetic mechanism of disease progression. With diabetes antioxidant properties worsen significantly.*

Objective. *The aim was to investigate the effect of L-arginine on oxidative stress parameters and microalbuminuria in type 2 diabetes mellitus and chronic kidney disease patients.*

Methods. *In total, 57 patients with type 2 diabetes mellitus and chronic kidney disease and 30 healthy individuals (control group) took part in the study. The patients were divided into 2 congruent groups. The 1-st group of patients (n=33), in addition to standard therapy, received L-arginine 4.2 g intravenously for 5 days, after that they took it 1.0 g orally three times a day during meals for 1 month. The second group of patients (n=24) received a standard therapy.*

The concentration of lipid peroxidation products was measured by a spectrophotometric method. The determination of MAU was carried out in morning portion of urine immunological semiquantitative using test strips.

Results. *Significant improvement in indexes of lipid peroxidation was observed in both groups after therapy ($p<0.01$), but in patients treated with L-arginine it was more expressed ($p<0.01$). The standard therapy did not significantly affect the level of MAU ($p>0.05$). The patients treated with L-Arginine, showed a significant reduction in MAU ($p<0.01$).*

Conclusions. *L-arginine facilitates the correction of lipid peroxidation processes and reduces the severity of microalbuminuria in patients with diabetic kidney disease that slows down its progression.*

KEY WORDS: **diabetes mellitus, chronic kidney disease, lipid peroxidation, microalbuminuria, L-arginine.**

Introduction

In recent decades a number of patients with diabetes mellitus (DM) is increased, the patients with type 2 diabetes mellitus are a significant share of them [1, 2, 3].

Diabetic kidney disease (DKD) is one of the severest complications of diabetes; it is registered in 20–30% of patients with diabetes. Among the patients, who receive renal replacement therapy, the share of people with DKD is about 40–50% [2, 4]. It is established that 10-20% of patients with diabetes die from chronic renal failure [4, 5].

Microalbuminuria (MAU) is one of the first signals of DKD. It is not only the symptoms but also an important pathogenetic mechanism of progression [6, 7]. According to recent studies, MAU is considered to be an independent predictor of progressive renal disease [6, 7]. Despite the fact that MAU is regarded as a risk factor, in the timely diagnosis and adequate treatment it can achieve the level of recourse normoalbuminuric [6, 8]. But in 30–45% of patients over 10 years old the disease progresses to proteinuria. On the other hand, in 20% of patients over 20 years old a terminal kidney failure is developing either [6, 7].

With diabetes antioxidant properties worsen significantly. The activity of antioxidant defence in the tissues is reduced; a lipid peroxidation is disordered that has repeatedly been proven in experimental diabetes [9, 10]. It is important to reveal as soon as possible the disorders of pro- and antioxidant system for diagnosis and monitoring of the health state. The timely correction of changes can prevent the progression of vascular complications of diabetes [9].

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The aim of our research was to investigate the effect of L-arginine on oxidative stress parameters and microalbuminuria in type 2 diabetes mellitus and chronic kidney disease patients.

Methods

A total of 57 patients with type 2 diabetes mellitus and I-V stages of chronic kidney disease took part in the study. There were 27 (47%) men and 30 (53%) women among them. The average age of the patients was (58.7±1.2) years old. The average duration of diabetes is (11.4±0.9) years. The average illness period of CKD is (2.5±0.3) years. The average weight of patients was (81.7±1.4) kg. 30 healthy individuals composed the control group.

The diagnosis was established by the Second National Congress which was adopted as Nephrology classification of diseases of the urinary system in 2005. The stage of CKD was determined by glomerular filtration rate (GFR), defined by the formula CKD-EPI [11].

The concentration of thiobarbituric acid *reactive* substances (TBARS) was determined in the blood serum by a spectrophotometric method [12]. We also evaluated the concentrations of glutathione (SH-group) [13], catalase [14], superoxide dismutase (SOD) [15] in the blood plasma.

The determination of MAU was carried out in morning portion of urine immunological semiquantitative using test strips CLINITEK® Microalbumin 2 Strips (Germany) by means of the unit CLINITEK Status® + Analyser (Germany). MAU was diagnosed at the level of urinary albumin excretion within 30–300 mg/day, normoalbuminuria – less than 30 mg/day.

The patients with DKD were divided into 2 congruent groups: the 1-st group of patients (n=33), who, in addition to standard therapy received L-arginine 4.2 g intravenously for 5 days, after that they took it 1.0 g orally three times a day during meals for 1 month. The second group of patients (n=24) received a standard therapy. The results were compared with those of the healthy controls (n 30).

The data were subjected to statistical research processing. For this purpose the application package Statistica (StartSoft USA, v.12) was used: the method of parametric and nonparametric statistics, Spearman rank correlation to determine the connection between the studied parameters. Statistically significant differences in $p<0.05$ are considered.

Results

Analyzing the results, it was found outthat 100% of patients with DKD have lipid peroxidation disorders (Fig. 1).

The average level of MAU in the DKD patients before treatment is in 25.6 times higher than in the control group (Fig. 2).

We have analysed the findings of MAU, depending on the method of treatment in DKD patients groups before and after treatment (Table 2).

Discussion

The average levels of TBARS and SH-groups in the patients with DKD before treatment were significantly increased compared with the corresponding values in the control group ($p<0.01$). Significantly reduced SOD and catalase activity were detected in the observed DKD patients before treatment compared with the control group ($p<0.01$). No significant difference in lipid peroxidation indexes was noted between two DKD patients groups before treatment ($p>0.05$). Significant improvement in lipid peroxidation indexes was observed in both DKD patients groups after therapy ($p<0.01$), but in the pa-

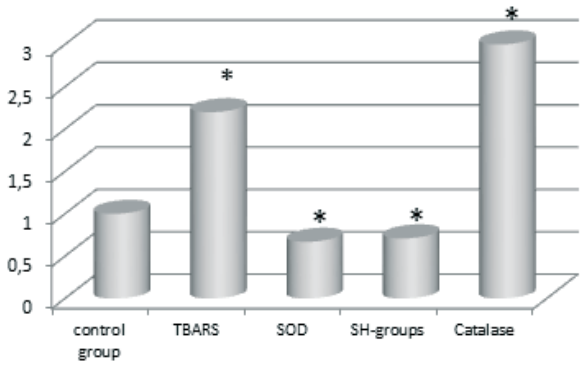

Fig. 1. The level of lipid peroxidation indexes in DKD patients before treatment and control groups.
* – The difference between the control group and DKD patients group is significant ($p<0.01$).

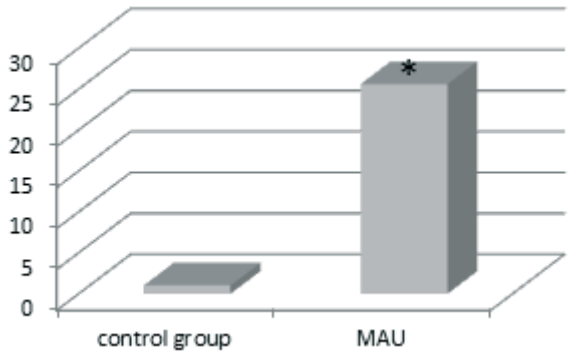

Fig. 2. The level of MAU in the DKD patients before treatment and in the control groups.
* – The difference between the control group and the DKD patients group is significant ($p<0.01$)

The effects of treatment on lipid peroxidation indexes are presented in Table 1.

Table 1. The dynamics of lipid peroxidation indexes in DKD patients before and after treatment depending on different treatment regimens, M±m

Indexes		Groups		p		
		Group I (n=33)	Group II (n=24)	p_1	p_2	p_3
TBARS, mkmol/l	Before treatment	6.26±0.20	6.20±0.13	p>0.05		
	After treatment	3.81±0.27	5.27±0.46	p<0.01	p<0.01	p<0.05
SOD, %	Before treatment	41.76±0.68	41.45±0.66	p>0.05		
	After treatment	55.61±1.42	43.21±1.18	p<0.01	p<0.01	p>0.05
SH-groups, mlmol/l	Before treatment	42.59±1.23	43.71±2.08	p>0.05		
	After treatment	57.05±1.98	51.14±1.65	p<0.05	p<0.01	p<0.05
Catalase, %	Before treatment	53.74±2.16	51.46±1.18	p>0.05		
	After treatment	31.39±2.80	47.25±2.88	p<0.01	p<0.01	p>0.05

Notes:
1. p_1 – significant difference in performance between groups before and after treatment.
2. p_2 – significant difference in performance in the first group before and after treatment.
3. p_3 – significant difference in performance in the second group before and after treatment.

Table 2. The dynamics of MAU levels, depending on different treatment regimens, M±m

MAU mg/day	Groups		p		
	Group I (n=23)	Group II (n=24)	p_1	p_2	p_3
Before treatment	180.7±19.9	188.6±15.6	p>0.05		
After treatment	51.4±15.9	149.2±15.5	p<0.01	p<0.01	p>0.05

Notes:
1. p_1 – significant difference in performance between groups before and after treatment.
2. p_2 – significant difference in performance in the first group before and after treatment.
3. p_3 – significant difference in performance in the second group before and after treatment.

tients treated with L-arginine it was more expressed (p<0.01).

The standard therapy did not significantly affect the level of MAU (p>0.05). A significant reduction in MAU (p<0.01) was proved in the patients treated with L-Arginine.

The role of oxidative stress in the pathogenesis of diabetes has been examined repeatedly in the experimental models. Renal cortical superoxide production is increased in the early stage of experimental diabetes that leads to vasoconstriction of afferent arteriole [16]. Recent studies in experimental models of diabetic nephropathy indicate that vascular synthesis of NO protects from progression of renal lesions in diabetes [17]. L-arginine is a semi-essential amino acid and also the main source for the generation of NO via nitric oxide synthase [18].

Recent study demonstrated that L-arginine supplementation in type II diabetic rats was beneficial by preserving glomerular filtration rates, presumably via increased renal endothelial nitric oxide synthase levels, that leads to renal vasodilation [19].

In our present research L-arginine supplementation, which stimulates nitric oxide synthesis, causes a pronounced improvement in lipid peroxidation indexes and a significant reduction in MAU in the patients with DKD.

Conclusions

In patients with DKD, the decrease in activity of SOD and catalase and the increase of TBARS

and SH-group parameters is observed that proved a lipid peroxidation disorder.

L-arginine in the standard therapy facilitates the correction of lipid peroxidation processes and reduces the severity of microalbuminuria in patients with diabetic kidney disease that slows down its progression.

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NEURODEVELOPMENTAL CARE OF PRETERM INFANTS AND ITS KEY ELEMENTS

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During the past few decades, advancements in the perinatal and neonatal intensive care have led to a significant survival of premature infants. However neurodevelopmental outcomes remain the major issues of concern. Developmental care is an approach that aims to reduce the mismatches between the extra- and intrauterine environment, decrease the stress of preterm newborns in neonatal intensive care units, and thus promote optimal neurobehavioral development of the infant.

The Newborn Individualized Developmental Care and Assessment Program (NIDCAP) model was developed as a clinical framework for the implementation of developmental care. The model focuses on detailed reading of each individual infant's behavior. By observing the infant during a routine assessment (before, during and after) and a detailed description of his/her behavioral responses, a professional can assess the ability of the infant's immature nervous system to tolerate the environment and care manipulations. Such evaluation will enable one to determine the adequacy of the environment and care to meet the infant's opportunities and needs, with subsequent corrections and adaptation. NIDCAP's aim is to support the infant in his/her interaction with the environment as an active participant in caregiving within a family.

With the help of NIDCAP approaches in neonatal care medical staff will study how to read and interpret infants' behavior, thus hearing their voices.

KEY WORDS: **developmental care, NIDCAP, preterm infants.**

Introduction

Today the number of premature births is increasing. According to the World Health Organization (2012), every year about 15 million babies are born prematurely – more than one in 10 of all babies born around the world, affecting the families around the world. In developed countries of Europe and USA the incidence varies from 5 to 12% [1, 2, 3], and may reach 40% in less developed regions [2].

During the past few decades, advancements in the perinatal and neonatal intensive care (the use of antenatal corticosteroid therapy, surfactant therapy, and improved ventilatory techniques) have led to a significant survival of premature infants with gestational age less than 32 weeks [4]. The percentage of newborns with gestational age less than 32 weeks (extremely preterm and very preterm ones) is 1–2% in developed countries [1].

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However, despite the positive results of survival, morbidity and complications associated with premature birth are growing rapidly [2, 3]. Preterm babies meet with a large number of negative factors, since the beginning of their extrauterine life passes in the NICU (neonatal intensive care unit) environment, where such factors as light, noise, highly advanced non-invasive and invasive manipulation and pain dramatically affect the whole immature organism, resulting in early short-term and long-term complications.

Neurodevelopmental outcome has become a benchmark in determining the effectiveness of medical neonatal care [5]. The incidence of disability and neurodevelopmental problems among survivors of neonatal intensive care remains high and problematic [2, 6, 7]. Severe neurological complications (cerebral palsy, moderate to severe mental retardation, epilepsy, poor motor skills, sensorineural hearing loss, blindness) still remain consistently high. Moreover, by the age of 8 years, over 50% of very low birth weight preterm children require special

educational services and 15% have repeated at least one grade in school [3, 8, 9].

However, these are not the only neurological problems. The implementation of extended follow-up monitoring, using modern methods and scales of neurological evaluation allow us to identify мовшire and more neuro-developmental disorders in children. These disorders include learning disabilities, low average IQ scores, attention deficit, hyperactivity disorders, neuropsychological deficits, visual motor disintegration, executive function difficulty, varying temperament difficulties, language delays, emotional problems and regulatory disorders [5, 8]. 50–70% of very low birth weight premature infants suffer from these dysfunctions [3, 10].

In order to provide not only survival, but to optimize long-term health and development, it is necessary to know the features of brain development of the fetus in the intra-uterine environment and of the premature baby in the extrauterine space. The development of the brain of the full-term infant takes place in utero where the infant resides in a temperature-stable environment, receives all the nutrients, undergoes circadian rhythm, and most importantly, feels maternal presence and permanent protection [11, 12]. Prematurely born infants are deprived of all these favorable conditions in the most critical period when their brain is growing and developing most intensively [13]. At the same time, they are exposed to excessive manipulation in a high-technology medical NICU environment. The mismatch between the preterm infants' need that are necessary for brain development, and the realities of intensive care units may cause serious problems of neuro-physiological, psycho-emotional and psychosocial development.

Developmental care is an approach that uses a range of medical and nursing interventions that aim to reduce the mismatches between extra- and intra-uterine environment, decrease the stress of preterm neonates in NICUs, and thus promote optimal neurobehavioral development of the infant.

Review

The framework of developmental care views preterm infants as fetuses who find themselves too early and unexpectedly in a technologic hospital environment instead of the evolutionarily promised mother's womb [3, 14]. Behavioral individuality of each infant is the basis of neuro-developmental care. Each infant is seen as an active participant in all elements and at all stages of care. An infant's behavior provides the best information to determines the care management [14, 15].

The Newborn Individualized Developmental Care and Assessment Program (NIDCAP) model was developed as a clinical framework for the implementation of developmental care. NIDCAP is a comprehensive program which includes a behavioral observation methodology and creation of individual developmental caregiving support of the infant's own developmental goals [12]. The model focuses on detailed reading of each individual infant's behavior [2]. By observing the child during a routine assessment (before, during and after) and a detailed description of his/her behavioral responses, medical staff can assess the ability of the infant's immature nervous system to tolerate the environment and care manipulations. Such evaluation will enable one to determine the adequacy of the environment and care to infant's opportunities and needs, with subsequent corrections and adaptation [2, 15].

The infant's ability to regulate and control the behavior occurs within continuous interaction with the environment (whether womb, NICU, home conditions) and is expressed through the infant's five systems: autonomic (physiological), motor, state, attention/ interaction and self-regulation. These systems are continuously interacting with one another. Each system influences and supports the other, and interacts with the environment. The autonomic system functioning is manifested in an infant's breathing quality and rate, color fluctuation, and visceral stability or instability. The functional state of motor system is estimated by the position, muscle tone and movement patterns of the face, trunk and extremities. State organization is observable in an infant's range of available levels of consciousness, their robustness and modulation of the available states, and the patterns of transition from state to state. The activity of attention/interaction system is manifested by infant's look, facial expressions and the ability to interact [3, 12, 15]. The self-regulation system is manifested in infant's ability to re-balance and bring into harmony the other systems when they have moved out of balance [2].

NIDCAP methodology was founded by Heidelise Als (psychologist from the Harvard University) in 1986 and is based on the Synactive Theory of Development. Heidelise Als has been a pioneer in helping medical staff and parents to understand how to "read" preterm infant's

behavior, especially those who need intensive care [16].

The synactive theory of newborn behavioral organization and development (synaction n., or synactive adj. (from the Greek syn "together" and the Latin actio "action," resulting in "together in action"). The synactive theory of newborn behavioral organization and development suggests that development of the human fetus, and later newborn, proceeds through the constant balancing of approach and avoidance behaviors, leading to a continuous interaction of the above mentioned subsystems and their increasingly defined delineations within the organism, and the organism's interaction with the environment at large [12, 17].

The synactive theory of infant development provides that there is no possibility for development without stimulation, but there is also no harmonious development if the stimuli lead to disorganization and stress [15]. Sensory inputs are the important and necessary parameters in fostering central nervous system development [3]. Inappropriate stimuli penetrate into all systems and destroy them, while adequate and timely stimuli promote and improve the infant's growth and development [11, 15]. The infant's response to any stimulus manifests in signs of adaptation of the foregoing systems when he or she tolerates it, or in maladaptive signs if the infant is unable to cope. The infant is able to tolerate stimuli when they are appropriate in timing, complexity and intensity in relation to the infant's thresholds of functioning. If the stimuli are too intense, complex or inappropriately in time, infant either has strategies to move away from inputs, avoid them, or suffer from them. Such behavior is considered a stressful situation for an infant [2, 12]. The aim of the NIDCAP is to support the infant in his/her growing tolerance to stimuli, and to minimize stressful events and manipulations that require energy consumption, use of calories and sometimes break the physiological homeostasis [3]. Each caregiver must recognize that for the unstable or fragile infant the best form of sensory input may necessitate a plan of reduction of surrounding stimuli [18].

Signs of autonomic system adaptation in the infant are: stable color, regular breathing and heart rate within physiological ranges and stable digestion. Self-regulatory balance of motor system is reflected by the appropriate muscle tone, harmonious and smooth movements, softly flexed posture with flexed arms and legs; hands near the face, around the mouth or inside it [12, 15]. Sleep and waking cycles develop in time to become differentiated [15, 19]. The infant actively looks for a source of stimulation and visual contact with a person who cares for him/her, the face is open, and the infant has attentive look. This stability of the systems manifests in smooth function and reflects intact organization and central nervous system control [3].

Signs of autonomic system stress in the infant are: skin discoloration (pale, marble, red, cyanotic), breathing disorders (apnea, tachypnea) with or without desaturation, unstable heartbeat (bradycardia or tachycardia), functional gastrointestinal disorders (hiccups, regur_ gitation, vomiting) and neurological disorders (tremors, convulsions, yawning, sighing). Motor signs of stress include: muscle hypotonia, extension of the trunk (arching), ourward movements of limbs (splaying, airplanning, saluting, sitting on air) and tongue extensions. Sleep and waking cycles are not proper differentiated, with the infant avoiding stimulation and visual contact, there is lack of visual concentration. In general, extension behaviors are thought to reflect stress, and flexion behaviors are thought to reflect self-regulatory competence. Diffuse behaviors are thought to reflect stress, and well-defined behaviors are thought to reflect regulatory balance [12, 15]. Such stress reactions may indicate that environmental stimuli of light or noise or even caregiving events such as turning the infant or changing the diaper, may be overwhelming [3]. To overcome these stressful situations infant spends the energy that he needs to maintain his homeostasis [3, 15].

Another behavioral scenario is possible when the infant cannot respond to environment or handling stimuli. The infant may lie in the bed, limp and flaccid, unable to put any energy into responding to caregiver; or just develop minimal ranges of responsiveness [15, 17].

The ability to regulate or control the infant's autonomic, motor and state organization is a requirement of early development and it is called self-regulation [20]. Early self-regulation is accomplished in the mother's body as a fetus, and continues over the course of the first three years of life after birth. The first tasks of self-regulation involve the regulation of physiological function as breathing, heart rate regulation, maintenance of visceral control, body temperature and homeostasis and day–night cycles regulation. Later the infant learns to calm himself and relax even after mild stress. As an infant matures in the context of daily interactive care from consistent primary caregivers, self regula-

tion becomes the task of learning to control motor behaviors, sleep–wake cycles, attention and interaction [3, 15, 20, 21]. Eventually, these capacities merge into emotional regulation. The premature baby often needs external assistance in self-regulation. Parents and caregivers become the infant's "co-regulators" and support infants by accurately reading and interpreting the infant's behavioral signals [3].

How assess and interpret the child's behavior according to NIDCAP? Developmental specialists observe an infant's behavior weekly or every other week throughout the hospitalization, starting with the phase of the infant's initial stabilization. During each observation, the developmental specialist systematically records an infant's behavior for approximately 20 minutes before a planned medical or nursing caregiving interaction and continues to observe throughout the duration of the interaction and for approximately 20 minutes after it. Ninety-one behaviors, including autonomic (breathing, heart rate, color changes and visceral signs), motor (postures, muscle tone fluctuations and movements) and state organization behaviors (levels of arousal, patterns of transitions between states, and clarity and robustness of sleep and awake states) are monitored every 2 minutes. Behaviors are conceptualized as regulatory and as stress according the foregoing signs of adaptation and disadaptation and are interpreted as indices of the infants' current strength and vulnerabilities respectively [14].

After the observation the developmental specialists write descriptive neurobehavioral reports and suggestions, which describe the infant's strengths, current sensitivities and apparent goals and thresholds to stress, and the infant's self-regulatory efforts at rest and during care manipulations. It helps to structure caregiving procedures to the infant's sleep/wake cycle, adapt or modify the environment and care according to the child's needs with the aim to maintain the infant's well-regulated behavioral balance in an effort to promote the infant's strengths and simultaneously to reduce the infant's self-regulatory vulnerability [2, 14]. As the infant matures, these recommendations are modified appropriately [22].

Key elements for implementation of individualized developmental family-centered care NIDCAP : 1) provision of sensitive care and handling for baby, 2) creation of a calm NICU environment, 3) parental involvement in infant care, their support, 4) presence of infant developmental specialist [3].

Provision of sensitive care and handling for the infant based on an accurate reading of infant behavioral signals and respect for the message that the infant communicates, along with recognition that the infant actively participates in all aspects of neonatal care [3].

a) Structure the infant's 24-hour day in accordance with the individual infant's sleep-wake cycles, state of alertness, medical needs and feeding competence. It may be necessary to divide or to combine procedures (eg. medical examination and care manipulations), to offer pauses according to the child's behavioral responses or to perform care with two persons. This approach provides infant's tranquility and thus promotes his/her growth [15].

b) Non-nutritive sucking suppors infant's physiological stability, improves sleep/waking cycle and reduces time of hospitalization [23].

c) Infant's flexed position reduces stress reactions, improves self-regulation, physiological stability and sensory stimuli tolerance and prevents the skeletal deformities [24, 25].

d) Positioning in the "nests" provides flexed position of the whole body with pelvic support (physiological position of the fetus), which prevents the formation of pathological extension position and hypertonus [24, 25].

e) Swaddling reduces physiological distress, maintains temperature homeostasis, improves motor development and optimizes the sleep/waking cycle [26].

Creation of a calm NICU. It is necessary to create a supportive, gentle environment for the most fragile infants (pic. 1, 2)

a) Noise reduction (reduction of alarm volume, decreased ringtone volume of telephones, modification of individual and collective behaviors – quiet conversation, avoiding the noisy gestures, individual rooms, installation of a sound measurement device. Sound levels shall

Pic. 1. Incubators in the NICU are covered to reduce the light and noise, to simulate day-night cycle.

Pic. 2. The device for monitoring the volume of sounds in hospital wards (green – normal, yellow – loud, red – noice).

not exceed 45 dB and hourly L10 of 55 dB; transient sounds should not exceed 70 dB; equipment in NICU should have noise levels of 40 dB [27, 28].

b) Reduction of the light level (avoiding direct illumination into the infants'eyes, except during examination, incubator cover adapted to the infant's awakening, individual illumination lamps at each incubator, providing circadian rhythm with decreasing light intensity at night, access to daylight) [3, 27].

Parental involvement in infant care, their support.

a) Early parental involvement in caregiving and participation in all care with day-and-night access in the NICU [15].

b) Skin-to-skin contact with mother or father, including children who need invasive and non-invasive respiratory support. Such contact has significant advantages for both the infant (temperature homeostasis, respiratory stability with a decrease in apnea and bradycardia, calm and lasting sleep, feeling of safety, positive dynamics of body weight, decreased risk of nosocomial infections) and mother (improving of lactation, stress reducing, sense of paternity) [15, 29, 30], (pic. 3, 4, 5, 6, 7).

Pic. 4. Kangaroo mother care of the ELBW infant with nCPAP.

Pic. 3. Kangaroo mother care ("skin-to-skin"contact) of the preterm infant with nCPAP.

Pic. 5. Kangaroo mother care of the twins.

Pic. 6. Kangaroo father care of triplets.

Pic. 7. Relaxing in skin-to-skin contact after breast-feeding.

Developmental care supports the relationship between the child and the family [12]. For the human species, parents are the familiar, constant and steady connection in an infant's life. They assure their infant's safety, and assure their child's development of trust and as a whole person [2]. The core principle of NIDCAP is the collaboration with each infant and the infant's parents as the primary caregivers and co-regulators; and the main goal of developmental care in the NICU and throughout the hospital stay is to improve child and family comfort and outcomes.

The presence of infant developmental specialist. The developmental specialist conducts regular NIDCAP observations and staff/parent teaching, provides consistency of developmental support to medical staff, family and the infant, articulates and adjusts the NICU

in the accordance with the developmental needs of the infant [3].

Positive aspects of developmental care. The studies have documented the beneficial effect of NIDCAP in terms of shorter intensive care and overall hospital stay, decreased time on the ventilator, decreased oxygen days, shorter duration of parenteral feeding, shorter transition periods to full enteral feeding, better average daily weight gain, lower total hospital charges and effect in enhancing neurodevelopmental outcome [11, 31- 4]. Some research has also documented that the NIDCAP approach enhances brain structure and function when measured by sophisticated medical techniques such as EEG and MRI [35].

NIDCAP also has significant benefits for parents. The studies of H. Als and other developmental specialists show the reduction in parenting stress and enhanced parent perception of the infant [31, 36, 37]. Except for short-term positive effects, neonatal care according the NIDCAP has also long-term effects on the infant's neurological development, behavior and mother–child interaction [34, 36].

Conclusions

As survival of high risk infants has continued to improve, greater emphasis has now been placed on improving neurological outcomes and quality of life. The implementation of individualized developmental care encourages the shift from a protocol-based task and schedule oriented framework of NICU care to an individualized and relationship-based framework of care. The introduction of NIDCAP in the neonatal departments will reduce the mismatch between intra-uterine environment and NICU by taking into account the individual infant's current thresholds of behavioral organization, diminishing stress, and supporting each infant's strengths and competencies. With the help of NIDCAP approaches in neonatal care all staff will study how to read infants' behavior, to hear their voices and understand them. Our smallest and most fragile patients should feel comfort and a sense of security that are so important for their healthy development.

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CHRONIC MYCOTIC-ASSOCIATED SURGICAL NAIL PATHOLOGY COMPLICATED WITH INGROWN NAIL (NAIL INCARNATION): THE ANALYSES OF CLINICAL CASES AND COMPLEX TREATMENT

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Background. *Conservative treatment of secondary recurrent unguis incarnatus are not very effective and Dupuytren's method, Emmert-Schmiden surgeries etc. are very traumatic, disfigure nail bone, distort anatomic and functional unity of a nail.*

Objective. *The aim of our research was the optimal sequence of holiatry, surgical moving away of nails, local and system therapy after excision of the staggered nails in case of destructive onychomycosis complicated by secondary ingrown nail for some patients with complicated mycotic defeat of nails. Clinical options of surgical treatment were clarified; morphologic changes were studied; causes of unsatisfactory outcomes of chronic pathology complex treatment were analyzed for prospective approaches to preventing of relapses.*

Methods. *The analysis justifies the feasibility of establishing of predictive relationships between clinical variants of chronic purulent necrotic infections and combined comorbidity. System therapy by itraconazole before operative treatment (basic onychial defeats sanation) and in a postoperative period was carried out. Types of operative treatment applied may be divided into five statistic groups.*

Results. *Over a five-year period (2010-2015) 436 unguis incarnates diagnosis (325 cases of incarnated onychomycosis) in 259 men and 177 women aged 12-67 were performed, 38 of them with incarnated onychogryphosis had diabetes mellitus and 24 had metabolic syndrome. Analysis of subonychial scraping allowed stating the prevalence of red trichophytia in 31% of cases with the bacterial flora. Patients with combined pathology got 4-5 five-day system 'pulses' of 400 mg/day itraconazole therapy. In patients with primary advantage of providing low-impact methods of nail excision with access via onycholisation structure a nail extirpation and marginal matricectomy was carried out; dermatophytoms and stratification on a nail bed were deleted.*

Conclusions. *Type of onychectomy wound cytograms in the study group on the 10th day was defined as regenerative-inflammatory in 24.81%, regeneratory in 75.19% (p=0.031). The methods of surgical treatment of mycotical recurrent nail incarnation have been improved considering patho- and morphogenetic properties of destructive onychomycosis.*

KEY WORDS: **destructive onychomycosis; secondary recurrent ingrown nail; antimycotic therapy; surgical nail removal.**

Introduction

Dermatophytes, infecting a nail matrix, were determined as the dystrophic changes of nail and a subnail hyperkeratinization and dermatophytoms [2, 3], surgical nail pathology, that compress a nail that is the driving nosotropic member of the secondary ingrowing and has an influence on curative tactics [4], in particular on the necessity of the surgical moving away [8, 21]. The pathogenetic links leading to increase of the probability of occurrence and development of feet mycosis and onychomycosis for diabetic patients include the pathology of cardiovascular and nervous systems [10], disruption of glycolysis, resulting in lower energy supply of skin cells and changes in metabolism, skin dysfunction, determining rapid progression and chronic mycosis [18, 20].

The objective was to study certain peculiarities of mycosis-associated pathology and treatment, including surgical removal of nails in patients with onychogryphosis and recurrent ingrown with underlying diabetes mellitus [20]. The results of determining of vitamins B_1, B_2, PP, B_6 and C in blood and their derivatives are analysed [22]. Some 50-year-old patients, who have high mycotic surgical nail pathology, have

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the endothelial dysfunctions and polyvitamin disturbances that are some factors from clinical corrections [10, 12, 22].

The study involves peculiarities of treatment, some indicators of lipid exchange of patients with diabetes mellitus [9, 10, 18, 20], metabolic syndrome accompanied by destructive onychomycosis and secondary nail ingrowth. Clinical options of in-patient and out-patient surgical treatment for nail ingrowth (mono-lesions; complicated and combined mycotic-associated processes and relapses) were clarified; morphologic changes were studied; causes of unsatisfactory outcomes of chronic pathology complex treatment were analysed for prospective approaches to preventing relapses.

If the infection involves the eponychium as well as the lateral fold, it is called eponychia [15, 17]. Its extension to the opposite side of the fingernail, which is uncommon, is called run-around abscess. In these cases, the paronychia is compressed along the nail edge, trapping the abscess. All procedures that successfully treat paronychia separate it from the hard nail [23, 24]. If the infection is limited to less than one-half of the eponychium, a single incision placed to drain the paronychium and to elevate the eponychial fold for excision of the proximal one-third of the nail is satisfactory. If the entire eponychium is involved, two incisions are required. The relevance of the problem of the ingrown nail (onychocryptosis, unguis incarnatus) in an urgent outpatient purulent surgery is caused by the increased frequency of its occurrence, chronicity, complications, not uncommon early and late postoperative relapses [8, 15, 17]. Conservative and orthopedic treatment of incarnatus surgical nail pathology are not very effective while Dupuytren's method, Emmert-Schmiden surgeries etc. are very traumatic, disfigure nail bone, [4, 5, 21, 24] distort anatomic and functional unity of a finger and in 2–20% of cases (depending on absence or presence of onychocryptosis and fungal agents) cause a relapse.

The clinical features and ways to optimize the treatment of patients with onychomycosis and destructive secondary ingrown nails are clarified in the article. The results of complex treatment of the patients with nail trichophytosis, associated with ingrown toenail, submitted according to dependence on a nail plate and eponycheal changes are presented in the publication.

The aim of our research is the optimal sequence of holiatry, surgical moving away of nails, local and system therapy after moving away the staggered nails in cases of destructive onychomycosis complicated by the secondary ingrown nail for some patients with the complicated mycotic defeat of nails.

Methods

Certain peculiar features of the clinical course and comprehensive treatment were studied, including surgical removal of the nails in patients with onychogryphosis associated with an ingrown nail (onychocryptosis, nail incarnation). Results of ingrown nail surgical correction were studied to improve the results of complex treatment. Over a five-year period (2010–2015) 436 unguis incarnates diagnosis (325 cases of incarnated onychomycosis) in 259 men and 177 women aged 12–67 were performed. In 182 patients late relapses of onychocryptosis were confirmed after previous surgeries at other clinics. Conservative treatment was recommended only at early stages of ingrowth. Removal of the affected nails was performed for patients with mycotic lesions (local and systemic fungicide therapies were used). Investigation of the morphogenesis of destructive aspect of the mycotic lesions was carried out. A variety of factors, aetiology, and pathogenesis of chronic purulent necrotic lesions of the foot, contributing to the occurrence, progression and recurrent course of these diseases, creates objective difficulties of diagnosis [14, 16, 19]. The analysis justifies the feasibility of establishing predictive relationships between clinical variants of chronic purulent necrotic infections and combined comorbidity [1, 25]. System therapy by itraconazole in operative treatment (basic onychial defeats sanation) and in a postoperative period was carried out [12, 25]. The applied types of operative treatment of surgical nail pathology may be divided into five main groups: 1 – Emmert-Schmiden type surgeries (marginal excision of nail plate and eponychia with marginal removal of the growing part via partial matricectomy); 2 – Dupuytren's type surgeries (onychectomy – complete removal of nail plate); 3 – Bartlett type surgeries (local tissue plastic reconstruction); 4 – marginal resection of marginal section of nail plate; 5 – Meleshevych surgery; 6 – our modifications (with previous block-type eponychectomy).

Results

Over the five-year period we examined and treated 98 patients of 52–86 years old with in-

carnated onychogryphosis (Fig. 1): 67 men (68.37%) and 31 women (31.63%) of the examined patients, 38 of them had diabetes mellitus and 24 had metabolic syndrome. Three variants of dermatophytoma are differentiated: front centre, with up to 25% of eroded nail – 45 cases, subtotal, from 25 to 70% (without capturing the growth plate) – 38 cases, total from 70 to 90% (with affected growth plate of the nail) – the other 15 cases. In all cases, dermatophytoma affected distal and central part of the nail bed. Analysis of subonychial scraping allowed stating the prevalence of red trichophytia [3, 4]: in 74% of cases it was associated with mold, in 26% of cases – with yeast fungi; in 31% of cases – with bacterial flora [1].

Conglomerate of nail plate and subungual hyperkeratosis and trichophytosis calcinated completely, forming onychogryphosis with deformation [6, 7] (Fig. 2) and secondary recurrent ingrown nail [15, 16, 22, 23].

In patients with onychomycosis, especially in severe destructive forms of subungual hyperkeratosis, large deterioration of microcirculation was noted. Rheographic prevailed spastic type curves ($p<0.01$) [9, 10]. Index of open capillaries was reduced by 31%. Patients in both groups mainly were affected with hallucis on left foot – in 188 people, the other – on right foot (133 patients), the presence of pathological ingrown nail plates of hallucis of both feet [12, 15, 17] and otherfingers were examined in other patients.

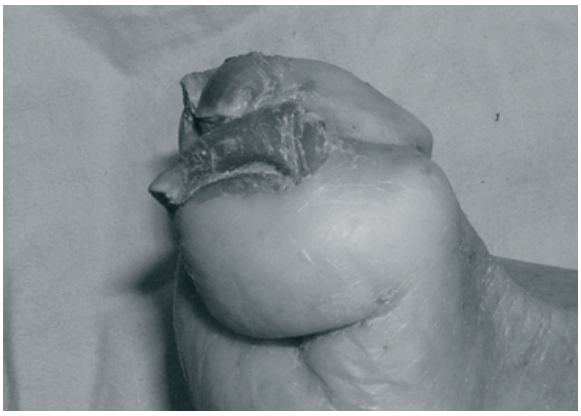

Fig. 1. Trichophytial polyonychomycosis, onychogryphosis. Clinical case. 81-year-old woman.

Fig. 2. Total nail dermatophytosis (Tr. Rubrum), polyonychomycosis. Big polyonychial gryphosis. Clinical case. 82-year-old man.

Mycotic associated hyponychial panaritium was diagnosed in 16 patients with onychogryphosis (16.33%), purulent paronychia was diagnosed in 11 patients (11.22%), the other 5 patients of this group suffered from eponychial abscess (5.10%). Patients of the main group underwent a three-day adjuvant systemic fungicide therapy: 400 mg itraconazole daily, during 4 days (the first 2 postoperative days) as pulse therapy. A similar dose at weekly intervals was carried out following five-day 2–3 cycle pulse [23, 25].

Removal of the affected nails for patients with polyonychomycosis was performed in successive stages at add-back of certain systemic 'pulses' with itraconazole. Patients with such combined pathology got 4–5 five-day system 'pulses' of 400 mg/day itraconazole therapy together with a simultaneous use of hepatoprotectors and correction of comorbid pathology [21–23]. Provided adequate surgical treatment, in addition to standard decompression stage (complete removal of the nail plate), contained antirecurrent component to prevent from repeated ingrowth. Along with antimycotic therapy and correction of comorbid pathology the following procedures were carried out: cutting pathologic eponychial tissues, hypergranulations and necroses [22, 24] (Fig. 3); removing nail plate with partial marginal ma-

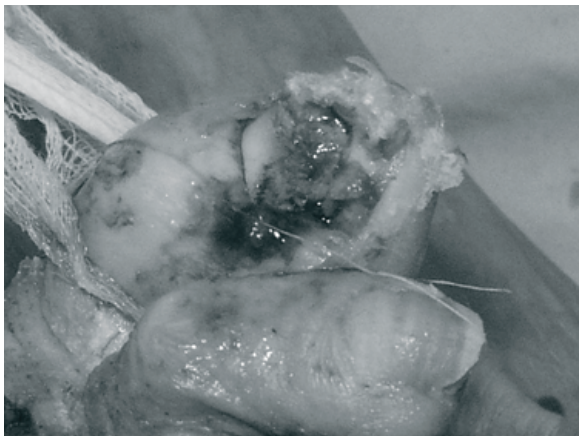

Fig. 3. Surgical nail pathology, subnail subungual hyperkeratosis and dermatophytosis, big dermatophytoma, hypergranulations and local necroses. Intraoperation photo. Trichophytial polyonychomycosis, incarnated onychogryphosis. Clinical case. 81-year-old woman.

trixectomy in the ingrowth area [2, 3, 20]. Non-invasive methods of nail excision and marginal nail resection were preferred for patients with diabetes mellitus.

During the five-year period we examined and treated 38 patients with onychogryphosis and diabetes mellitus type 2. We provide the research on the characteristic features of the pathological process to create the optimal scheme of complex treatment for patients with abnormal ingrowth of nail plate with underlying diabetes mellitus type 2 [9, 18].

23 patients with ingrown onychogryphosis and underlying diabetes mellitus, diabetic micro and macroangiopathy (prospective material, the treatment group), and onychogryphosis and recurrent incarnation of toenail (pathology being clinically dominant and manifesting through pain syndrome) and multiple destructive mycotic lesions of other nail plates of both feet: 14 men and 9 women, 55–80 years old were involved in the surgery. The other 15 people with onychogryphosis of the first toe (hallux) and fungus of other nails constituted the control group. The duration of clinically-manifesting nail mycotic process in all studied cases exceeded 5 years. Mycosis-associated acute purulent pathology [19, 21, 23] was confirmed in 13 patients (34.21% of the examined, 52% cases) of the treatment group and in 5 patients of the control group (13.16% of the examined, 30% cases) correspondingly. Systemic adjuvant pulse therapy with 400 mg itraconazole was applied during two days before the initial surgical treatment and during the first three days of postoperative period [19]. Remediation of other affected nails in order to prevent from mycosis reinfection was carried out with antimycotic amorolfine 5% lacquer and ciclopirox 8% nail lacquer solution [2, 11–13, 22]. Removal of other nails affected by hyperkeratosis with trichophytosis was performed through onycholysis by separate successive stages, where certain 'pulses' of therapy were supported with itraconazole [19, 21].

We prospectively examined 93 patients with metabolic syndrome [9, 10], associated with surgical nail pathology, destructive onychomycosis and secondary ingrown toenails. All patients underwent a comprehensive treatment of comorbid pathology, corrected using the systemic antimycotic therapy and terbinafine antifungal liniments, ciclopirox nail lacquer [11, 13] and surgical methods for removing nail plates [8, 9, 12, 22]. We studied lipid metabolism that manifested biochemical change indicators in the lipid profile, as well as their relations, which were significantly higher in both groups of patients (the main and control group), $p<0.01$ for both groups; we evidenced the positive correlation between the level of total cholesterol and leptin ($p<0.01$). The concentration of high-density lipoprotein cholesterol in patients of the main group was 1.12±0.06 mmol/L compared with patients in the control group – 1.07±0.06 mmol/L. The average content of nitrogen oxide in the study group (metabolic syndrome) was higher than in the healthy patients – 15.06±0.97 mcmol/L, $p<0.05$.

Significant decrease of HOMA-index of β-cell function and increase of HOMA-index of insulin resistance [18, 20] (10.21±1.9 in the treatment group and 4.12±1.12 in the control group, $p<0.01$) was detected in patients with underlying diabetes mellitus type 2 (the treatment group) with polyonychomycosis and trichophytosis onychogryphosis, and recurrent incarnation of the nail edge. High total cholesterol over 5.18 mmol/l was detected for all patients of the main group, i.e., 7.28±0.07 mmol/l, and 5.45±0.12 mmol/l in the half of the control group; the deviation of laboratory parameters of low and high density lipoprotein cholesterol was also confirmed.

Polyonychomycosis and big trichophytic subungual hyperkeratosis with secondary nail ingrowth [6-9] were diagnosed in the main group comprising 62 patients with type 2 diabetes mellitus (48 males and 14 females, aged 42-65). Metabolic syndrome was confirmed in the comparison group comprising 53 patients with arterial hypertension and complicated mycotic nail damage. The rest 276 patients constituted the control group. We studied biochemical findings, lipid blood spectrum: total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol; nitric oxide of blood plasma; leptin [9]. Lipid blood spectrum and nitric oxide level [10] were determined before and after pharmacotherapy along with continuous antihypertensive therapy (correction of intercurrent and comorbid pathology). The objective criterion of insulin resistance was the HOMA-IR index (the Homeostasis Model Assessment) involving glucose and insulin levels on an empty stomach divided by 22.5 coefficient. Patients of the main and the comparison groups with polyonychomycosis and trichophytic subungual hyperkeratosis with secondary ingrown nail experienced considerable decrease of HOMA-index of β-cells function and increasing HOMA-index of insulin resist-

ance (8.11±1.1 in the main group, 5.89±2.1 in the comparison group and 2.23±1.18 in the control group, p^1=0,01; p^2=0.01). Considerable positive correlation between all indicators of carbohydrate exchange was identified in groups comprising patients with type 2 diabetes mellitus: glucose and insulin (r=0.51; p=0.01), and the HOMA-index (r=0.70; p=0.01), and glycolyzed haemoglobin (r=0.75; p=0.001); insulin and the HOMA-index (r=0.73; p=0.01) and glycolyzed haemoglobin (r=0.65; p=0.01); the HOMA-index and glycolyzed haemoglobin (r=0.67; p=0.01). Considerable increase of circulating insulin was identified directly after surgical treatment (in the main group and the comparison group correspondingly 15.33±0.23 and 8.24±1.18 mkMO/l, p=0.01), the HOMA-index of insulin resistance (p=0.05) and the HOMA-index of β-cells function (p=0.05) in comparison with the control group with further tendency to some decrease in the process of treatment. We determined disturbances in lipid exchange, insulin resistance, lipid blood spectrum changes that were considerably higher in both groups of patients (the main and comparison groups), p=0.01 for both groups in comparison with the control group.

Discussion

On one hand the subungual hyperkeratosis and dermatophytosis caused compression of the central part of the nail, epionychium edges 'ingrew' to periungual walls thus recurrent ingrown nail was formed; on the other hand, constant compression caused destruction of central part of nail bed; this process was typical for 32 (84.32%) cases. Methods of surgical treatment of uncomplicated onychogryphosis [6, 7] and onychogryphosis complicated with recurrent nail incarnation [8, 15] were improved considering patho- and morphogenetic properties of destructive onychomycosis [16, 17]; removal of the mycotic affected nails of these patients should reasonably be conducted through onycholized structures with simultaneous removal of dermatophytoma, hyperkeratosis, and ingrowth areas with hypergranulation. Surgical treatment was performed according to the standard algorithm due to patholo gical eponychial changes in the patients with primary advantage of providing low-impact methods of nail excision with access via onycholisation structure and wedge resection of the nail [2, 20]. When combined incarnation of onychomycosis and acute eponychial abscess comply with disclosure abscess, excision of abnormal tissue eponychial hypergranulation and focal necrosis, and removal of the nail plate, enlarged partial marginal matrixectomy in the ingrowth area are performed. When combined with onychomycosis and nail incarnation acute eponychial abscess, an autopsy ulcer, excision of abnormal tissue eponychial hypergranulation and focal necrosis, and removal of the nail plate, enlarged partial marginal matrixectomy in the ingrowth area are made. Other patients (the control group) underwent a typical nail removal – operations such as Dupuytren's: complete removal of the nail plate under the guise of 'classical' pulse therapy by itraconazole and terbinafine [13, 21]; in 45 cases this intervention combined with simultaneous excision of the modified cuticle and plastic – operations such as Bartlett (plastic local tissue) and Meleshevich.

The processes of destruction of the nail in patients with trichophyton onychomycosis and type 2 diabetes mellitus / metabolic syndrome is much faster and is characterized by a more pronounced morphological variants of mycotic nail destruction that determine the occurrence of secondary ingrowth and attachment with intercurrent flora with the emergence of dermatophythoma with centres of decay and necrotic foci in the nail bed.

A nail extirpation and partial marginal matricectomy mechanical carving and diathermocoagulation with the further scraping off by the Folkman's spoon were carried out; dermatophytoms and stratification on a nail bed were deleted. Sanation of other nails for prevention of mycotic reinfection was carried out by ciclopirox nail lacquer [11]. Application of system enzymic protheolitical therapy allows to considerably improve the primary results of holiatry of the complicated subnail hyperkeratinization, diminish a perifocal edema and inflammation; stimulate the necrolitical and reparative processes in an operating wound that clinically shows up to the accelerations of its granulation and epithelization; accelerate cicatrization of surface, abbreviate the terms of temporal non-operability. For patients with type 2 diabetes mellitus (the main and comparison groups) with polyonychomycosis and dermatophytoms as well as secondary ingrown nail, subnail hyperkeratinization were performed for certain significant [20] reduction of the HOMA-index of function and the increase of the HOMA-index of insuline resistance. The chart of holiatry applied proved the effectiveness of treatment of bad resistance cases of destructive

polyonychomycosis by a subnail hyperkeratinization and secondary ingrown nail, in particular for patients with the type 2 diabetes mellitus [9].

Arguing that the removal of the nail plate in the patients with destructive onychomycosis with secondary incarnation nail advantageously carried out through onycholisation structure with simultaneous correction of pathological bed changes and cuticle, which in combination with antifungal treatment provides positive dynamics of regenerative type cytologic picture and shorter healing onychectomy wounds in 18 – 27 days to 12-25 days, with good early and long-term results. The type of transaction cytograms of onychectomy wounds in the study group on the 10th day of post-operative period is defined as the regenerative-inflammatory in 24.81%, as regenerative in 75.19% (p=0.031). In these embodiments, in the control group the indicators were respectively 53.12% and 46.88%. The advantage of regenerative option of cytological picture proves the correct choice of treatment strategy and accelerates wound healing after removing nail in patients of the main group. The factors that influence the occurrence and progression of incarnations, and disease recurrence after surgical treatment require further study [3, 4].

We have studied the results of onychocryptosis surgeries of surgical type 1 correction in 84 patients, type 2 – in 66 patients, type 3 – in the other 50 cases, type 4 – in 42 persons, type 5 – in another 27 patients, type 6 – in 56 patients (with the use of our modification of surgical treatment). Three types of trichophytosis have been differentiated: frontal central – with erosion of up to 25% of nail area, subtotal – from 25 to 70% (without touching upon growth area), total – from 70 to 90% (with affected growth area). In 65 mycotic trichophitis patients with secondary nail incarnation a standard itraconazole pulse therapy was applied. The presence of onycholytic focuses and degradation of hyperkeratotic areas which result in lamination of a part of nail plate proves the feasibility of performing low-trauma onychectomy for patients with trichophyton onychomycosis with secondary incarnation via onycholized structure with the single-stage sequential removal of dermatophyte and ingrowth areas with changed eponychial folds. Relapse causes after Meleshevych, Emmert-Schmiden, Bartlett surgeries were technical faults of surgical tools, intraoperative nail bed trauma, faults of postoperative anti-relapse treatments, surgical area trauma, wearing tight shoes, non-compliance with doctor's recommendations for correction of orthopaedic pathology, onychomycosis.

We believe that the less traumatic removal of nails through onycholysis should be prefered, particularly after such treatment, the patients with diabetes mellitus experienced healing time of operative wound (crust formation) during 16-23 days (average healing duration is 19 days) and had the indices tend to the control group; indices of the patients with diabetes and 'classical' nail removal (onicectomia) were normal in 24-30 days (average healing duration is 26 days), indices of the control group – in 14-22 days (average healing duration is 18 days).

Conclusions

In all cases of mycotic onychocryptosis (secondary ingrown toenail) the patients underwent a comprehensive treatment of comorbid pathology corrected by the systemic antimycotic therapy and terbinafine antifungal liniments, ciclopirox nail lacquer and surgical methods for removing nail plates, supplemented the eponychial resections and partial marginal matrixectomy.

System therapy of itraconazole before operative treatment (basic onychial defeats sanation) and in a postoperative period was carried out. Patients with combined pathology got 4-5 five-day system 'pulses' of 400 mg/day itraconazole therapy.

In patients with primary advantage of providing low-impact methods of nail excision with access via onycholisation structure a nail extirpation and partial marginal matricectomy mechanical carving and diathermocoagulation with the further scraping off by the Folkman's spoon were carried out; dermatophytoms and stratification on a nail bed were deleted. Sanation of other nails for prevention of mycotic reinfection was carried out by ciclopirox nail lacquer.

Methods of surgical treatment of uncomplicated onychogryphosis and onychogryphosis complicated with recurrent nail incarnation have been improved considering patho- and morphogenetic properties of destructive onychomycosis; removal of the mycotic affected nails of these patients should reasonably be conducted through onycholized structures with simultaneous removal of dermatophytoma, hyperkeratosis, and ingrowth areas with hypergranulation. Type of onychectomy wounds cytograms in the study group on the 10th day of the post-operative period was

proved as the regenerative-inflammatory in 24.81%, regeneratory in 75.19% (p=0.031).

The chart of holiatry applied proved the efficiency of treatment of bad resistance cases of destructive polyonychomycosis by a subnail hyperkeratinization and secondary ingrowing toenail; the less traumatic removal of nails through onycholysis should be preferred, particularly after such treatment the patients with the type 2 diabetes mellitus experienced healing time of operative wound (crust formation) during 16-23 days and the indices tend to the control group.

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PATHOGENIC MECHANISMS OF GENERALIZED PERIODONTITIS FROM THE POSITION OF POLYMORPHISM OF NUCLEAR TRANSCRIPTION FACTOR NF-KB1

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Background. *Periodontal diseases are a topical issue of contemporary dentistry because they are accompanied by severe morphological and functional disorders of maxillodental system; and are characterized by polyetiology and a number of metabolic disorders.*

The purpose of this study was to substantiate the pathogenic mechanisms of generalized periodontitis in relation to polymorphism of nuclear transcription factor NF- κB1.

Objective. *The aim of the study was to determine the genetic factors in the development of generalized periodontitis and the relationship of this parameter with immunohistochemical affiliation for cellular infiltrate of the lamina propria of gum at this nosology in young people. Hence, 2 groups were formed: I – control and II – observational.*

Methods. *Polymorphic gene section NF-κB1 was determined using the cells of buccal epithelium of the examined people by means of polymerase chain reaction. Collection of material was performed with sterile disposable dental brush, followed by the introduction of a reagent in ependorph with DNA Express reagent (LyTeh NPF, Russia). Genome deoxyribonucleic acid was isolated by DNA Express set (LyTeh, Moscow).*

Results. *The lack of correlation in this case indicates that no matter how parameters change, relatively major genotype (Del/Del) in this case is unchanged and the determining factor causes the development of generalized periodontitis, clinical picture of which is rapidly progressing.*

Conclusions. *Results of correlation analysis proved that genotype (Del/Del), as polymorphic variant of gene transcription factor NF-κB1, was significantly associated with the emergence of rapidly progressive periodontitis in young people.*

KEY WORDS: **gingival; generalized periodontitis; cells; nucleus; transcription factor.**

Introduction

Periodontal diseases are an urgent matter of contemporary dentistry because they are accompanied by severe morphological and functional disorders of maxillodental system; and are characterized by polyetiology and a number of metabolic disorders [1, 7, 12].

In case of inflammatory diseases of periodontal tissues, which are regarded today as polyetiological, such factors as genetic and non-genetic influence on each other with the effects that are not always clearly defined [10, 13]. In most cases, both types of factors influence the pathogenesis and severity of clinical symptoms.

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There is various systematics as well as clinical and morphological forms of generalized periodontitis [11] depending on aetiology, course of severity and immune response [14]. Polymorphic clinical features of this nosology are associated with different composition of subgingival microflora. The factors that affect antimicrobial response and new metabolic status of the organism are also important [6].

The problem of early diagnosis of generalized periodontitis, the development of effective measures of its prevention and treatment that is aimed at achievement of positive long-term results are topical matter of contemporary dentistry [2, 3].

It is established that various exogenous and endogenous factors and triggers [4] contribute to development of generalized periodontitis, but the influence of these factors is studied insufficiently [9].

Also, the established ideas and concepts [5] do not explain fully the existing differences in the identification of risk factors and progression of generalized periodontitis, its course, ambiguous outcomes under the same conditions. Due to the usual difference in the rate of generalized periodontitis progression in patients treated with the standard schemes, the biological heterogeneity of the disease and its possible complex causes are suggested.

In this regard, it would allow predicting the clinical course of generalized periodontitis in patients with this nosology. Therefore, studying the relationship of this disease with genetically determined factors should be recognized as perspective.

The purpose of this study was to substantiate the pathogenic mechanisms of generalized periodontitis from the position of polymorphism of nuclear transcription factor NF-κB1 that is based on patterns that determine the relationship between the genetic profile of an individual and the nature of cellular infiltrate of the lamina propria of gums in patients with generalized periodontitis.

Methods

To determine the genetic factors of generalized periodontitis development and the relationship of this parameter with immunohistochemical affiliation for cellular infiltrate the lamina propria of gum of this nosology in young people, 2 groups for observation were formed: I (control – 45 people with intact periodontium) and II (55 people – patients with generalized periodontitis).

Dental examination of 100 people was conducted in accordance with the standards of diagnosis and treatment of dental patients (the decrees of the Ministry of Health of Ukraine "On approving of the protocols of medical care in specialties "orthopedic dentistry", "therapeutic dentistry", "surgical dentistry", "orthodontics", "pediatric dentistry", "pediatric surgical dentistry" from 28.12.2002 №507 and "On approval of standards of medical care and quality of medical care" from 28.12.2002 №507).

Determination of polymorphic gene section NF-κB1 was performed using the cells of buccal epithelium of the examined people by means of polymerase chain reaction. Collection of material was performed with sterile disposable dental brush followed by the introduction of a reagent in ependorph with DNA Express reagent (LyTeh NPF, Russia). Genome deoxyribonucleic acid was isolated by DNA Express set (LyTeh, Moscow).

Polymorphic gene area NF-κB1 (rs28362491) was amplified by polymerase chain reaction. The final volume of the reaction mixture was 25 mkl and contained:

- specific oligonucleotide primers – 66 ng each,

direct – 5'-TGGGCACAAGTCGTTTATGA-3';
reverse – 5'-CTCGAGCCGGTAGGGAAG-3';

- 2,5 mkl 10 times buffering for amplification;
- 2 mM magnesium chloride;
- 0,2 mM deoxy nucleotide triphosphate (dNTP);
- 2,5 units. Taq DNA polymerase;
- 20–50 ng of genomic DNA.

In the tube containing these material 25 mg of mineral oil was placed.

Amplification was performed by means of Tertsyk thermocyclers (DNA Technology Ltd, Russia).

To identify alleles, the restriction analysis of amplicons using restriction enzyme PfIMI (SybEnzym, Russia) at 37 °C was performed.

Products cleavage of polymorphic gene section NF-κB1 was detected by horizontal electrophoresis in 2% agarose gel in a single TBE (50 mM Tris-H_3BO_3 and 2 mM EDTA, pH 8,0), for 2 hours at a voltage of 2 V per 1 cm of gel. pBR322/Alu I was used as a molecular weight marker of DNA. Gels were stained by etydiumom bromide followed by visualization of the results in the UV light.

According to the research objectives of the process of immunohistochemical studies, monoclonal antibodies CD-3 (clone SP7, DakoCytomation), CD-4 (clone 4B12, DakoCytomation), CD-20 (clone L26, DakoCytomation) were used. The results of of immunohistochemical studies visualization were carried out by En Vision system (DakoCytomation). The degree of expression of markers was determined by the colour intensity of specific patterns in brown colour. In some cases for structure differentiation, the obtained sections were stained additionally with Maher's hematoxylin. The degree of expression was evaluated individually for each marker and determined on at least (8-10) randomly selected fields of Biorex–3 BM–500T digital light microscope.

The results of clinical and complex morphological studies were analysed using a number of biostatistics methods. Statistical research was performed at the Department of Statistical Research of SIHE "I. Horbachevsky Ternopil State Medical University of the Ministry of Health of Ukraine". The data was processed by the licensed program Statistica (StatSoft).

To analyse the relationship of parameters that were studied (immunohistochemical profile of cellular infiltrate of the lamina propria of gums) the correlation coefficient (r) Spearman was determined. The correlation coefficient was considered statistically significant at $p<0.05$ [8].

The value of the correlation coefficient represents the degree of closeness between the values to a linear function, which correspond to the values ± 1 correlation coefficient. If $rxy>0$, the correlation is positive; it means that with increasing of one value, a second value increases in average. If $rxy<0$ correlation is negative, that is with increasing of one value, a second on average is decreased. If there is no statistical relationship between the values, the correlation coefficient is zero. The level of p-test (statistical significance) depends on the value of the correlation coefficient and the size of the experimental group for which the determination of the correlation coefficient is made. In the statistical processing of results, molecular genetic studies, in accordance with certain genotype (Del/Del), (Del/Ins), (Ins/Ins), as a basis for comprehension of the genetic structure of the population the law of genetic equilibrium by Hardy-Weinberg was applied. Due to this law, according to data on the frequency of recessive phenotype in the population that have homozygous genotype, the prevalence of polymorphic variants of NF-κB1 was calculated in the exemined groups. Statistical justification of probability differences of genotypes distribution was performed using χ^2 test adjusted for continuity by Yates [8].

Results

The molecular genetic study included a selection of polymorphic gene sites of nuclear transcription factor NF-κB1 cells of buccal epithelium by polymerase chain reaction.

The specificity of immunohistochemical markers expression on the plasmolemma of cells that formed inflammatory infiltrates made it possible to distribute them to the group and allocate three immunohistochemical profiles of infiltrates.

First immunohistochemical profile was characterized by prevalence of T lymphocytes infiltrates, which were expressed on plasmolemma of immunohistochemical marker CD-3 – multi-protein complex that was express on the surface of T-lymphocytes. The increased level of the marker expression on cells' plasmolemma, which appeared in a dark brown colour, was determined (Fig. 1).

Stated immunohistochemical profile correlated with the frequency of exacerbations 3 times a year, creating a statistically significant relationship with correlation coefficient ($r=0.58$, in $p<0.05$).

Second immunohistochemical profile was characterized by a predominance of T-lymphocytes in infiltrates that wereexpressed on plasmolemma by immunohistochemical marker CD-4 that was one of the proteins cluster of differentiation, monomeric transmembrane glycoprotein of superfamily Ig with a molecular weight of 55 kDa, which was the marker of T-helper cells.

In our research, a high level of the marker expression on the plasmolemma of cells, which appeared in a dark brown colour, was proved (Fig. 2).

The stated immunohistochemical profile correlated with the frequency of exacerbations once a year, creating a statistically significant

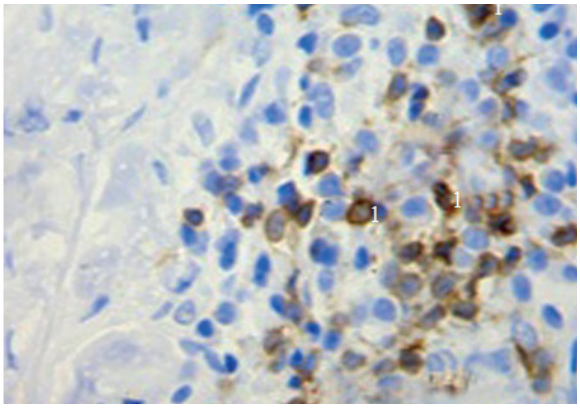

Fig. 1. The expression of CD-3 marker in the complex of cellular infiltrates in the lamina propria of gums in the course of generalized periodontitis. Immunohistochemical method, extra Meyer hematoxylin staining. Magnification: x 1000. 1 – high degree of expression.

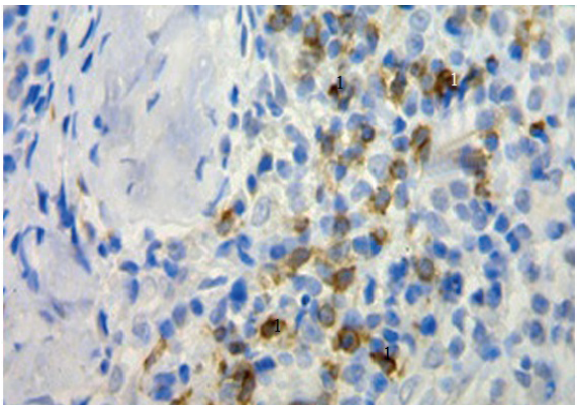

Fig. 2. The expression of CD-4 marker in the complex of cellular infiltrates in the lamina propria of gums in the course of generalized periodontitis. Immunohistochemical method, extra Meyer hematoxylin staining. Magnification: x 1000. 1 – high degree of expression.

relationship with correlation coefficient (r=0.51, in p<0.05).

Third immunohistochemical profile was characterized by a predominance of cells infiltrates that were expressed in plasmolemma by immunohistochemical marker CD-20 or B-lymphocyte antigen – a protein, co-receptor located on the surface of B-lymphocytes. It was established that in cell infiltrates of the lamina propria, high and moderate expression was determined in a dark brown and light brown coloration of cells (Fig. 3).

The stated immunohistochemical profile correlated between frequency of exacerbations 5 times a year, creating a statistically significant relationship, and correlation coefficient (r=0.94, at p<0.05). The correlation analysis of clinical dental and immunohistochemical research with determination of correlation coefficient (r) Spearman confirmed the correlation between frequency of exacerbations and immunohistochemical profile of an individual. The maximum value of the correlation coefficient (r=0.91) has CD-20 immunohistochemical profile and probable frequency of exacerbations 5 times a year. The determined correlation between CD-3 immunohistochemical profile and frequency of exacerbations – 3 times per year (r=0.58) and correlation between CD-4 immunohistochemical profile and frequency of exacerbations once per year (r=0.51).

Discussion

The stated presence of correlations between anamnesis, clinical indicators and character of CD- belonging to cellular infiltrate of the lamina propria of the gums in generalized periodontitis makes it possible to systematize generalized periodontitis in young people on clinical and morphological forms depending on the predominance of inflammatory infiltrates of T or B cells. The intensity of the course of inflammatory and destructive processes in periodontium is determined by reorganization of specific local immunity, which is provided by cellular and humoral components, which is reflected in the change of immunohistochemical profile of inflammatory infiltrates. The obtained results of correlation analysis enable to systematize generalized periodontitis relatively, in the examined people, on 'stable' with exacerbation rate once a year and CD-4 immunohistochemical profile; 'conditionally stable', with frequency of exacerbations 2–3 times a year and CD-3 immunohistochemical profile; 'progressive', with frequency of exacerbations 3–5 times a year and CD-20 immunohistochemical profile. Therefore, due to immunohistochemical features of cellular infiltrate of the lamina propria of gums in the course of generalized periodontitis it becomes possible to predict clinical course of nosology.

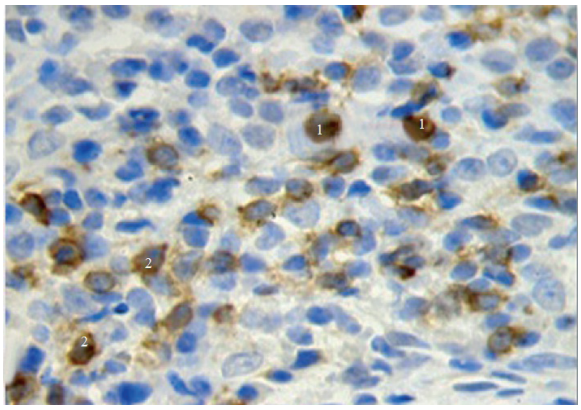

Fig. 3. The expression of CD-20 marker in the complex cellular infiltrates of the lamina propria of the gums during GP. Immunohistochemical method, extra Meyer hematoxylin staining. Magnification: x 1000. 1 – high degree of expression; 2 – moderate degree of expression.

The detailed analysis of molecular genetic studies proved that 30 (55%) patients with generalized periodontitis had genotype (Del/Ins) – heterozygote, another 16 people (29%) genotype (Ins/Ins) – homozygote, sized fragment of polymorphic area 94 bp. The most pronounced clinical manifestations of inflammatory and degenerative changes in periodontal tissues, characterised by clinical manifestation of rapidly-progressing periodontitis, was observed in 9 persons (16%) with genotype (Del/Del) – homozygote.

The results of correlation analysis, with definition of Spearman correlation coefficient (p<0.05), provides the data on the lack of correlation between the defined parameters in patients with polymorphic variant (Del/Del). The lack of correlation in this case indicates that despite the parameters changes (PMA, available or no concomitant somatic pathology, etc.), relatively major, in this case genotype (Del/Del) is unchanged and the determining factor causes the development of generalized periodontitis, clinical picture of which is to rapidly-progressing.

At the same time, in 30 people with genotype (Del/Ins) the direct correlation by Spearman (p<0.05) was identified between the immunohistochemical profile of an individual and available concomitant somatic pathology with correlation coefficient (r=0.60) between bad habit (smoking) and PMA index (r=0.17), which

indicates a direct role of these parameters in the emergence and development of generalized periodontitis.

It should be noted, the significant inverse correlation by Spearman ($p<0.05$) between PMA index and concomitant somatic pathology ($r=-0.40$) and immunohistochemical profile of an individual ($r=-0.37$) was evidenced.

The characteristic features of the defined correlation connections between parameters makes it possible to state that the registered clinical manifestations of generalized periodontitis in case of this polymorphic variants are not genetically determined, but possibly are caused by exogenous pathogens and local factors [12, 13] that initiate the development of induced generalized periodontitis, considering possible ways of activating transcription factor NF-κB1, which contributes to the emergence or enhances pathological responses in periodontal tissues (Table 1).

The absence of significant direct correlation by Spearman ($p<0.05$) between genotype parameters (Ins/Ins) should be noted; this leads to the assumption that the pathogenic mechanisms of generalized periodontitis in patients with this variant have polymorphic genetic component, but the features of clinical manifestations are more favourable and are characterized by chronic long course in comparison to the polymorphic variant (Del/Del). The results of correlation analysis provide an opportunity to offer systematics of generalized periodontitis due to the nature of inflammatory infiltrates of T or B cells and genotype (Table 2).

The control group consisted of patients with intact periodontal genotype (Del/Del): there were 5 people (11%); so the risk group for morbidity of generalized periodontitis have been developed and was recommend taking these individuals under medical supervision. 23 people (51%) with polymorphic variant of transcription factor NF-κB1 (Del/Ins) constituted a risk group with bad habits and concomitant somatic pathology that in the future would create preconditions for development of induced generalized periodontitis on the background of vascular disorders of the lamina propria of gums. 17 people (33%) with polymorphic variant of the transcription factor NF-κB1 (Ins/Ins) constituted a risk group in the case of impact of local adverse factors and current tooth-jaw anomalies and deformities (see Table 1).

Table 1. Distribution of polymorphic variants of gene transcription factor NF-κB1 in patients with intact periodontium of tissues and with generalized periodontitis

Characteristics of examined	Homozygotes (D/D)	Heterozygotes (D/I)	Homozygotes (I/I)
Patients with intact periodontium (45 persons)	5 (11%)	23 (51%)	17 (38%)
Patients with generalized periodontitis (55 persons)	9 (16%)	30 (55%)	16 (29%)

Note. The distribution of genotypes polymorphisms (Ins/Ins, Ins/Del, Del/Del) was determined in accordance with the law of genetic equilibrium Hardy-Weinberg; significant differences were defined by criterion χ^2 – adjustment for Yates continuity.

Table 2. Systematics of generalized periodontitis by 'immunohistochemical' and 'genetic' profile of the individual

T lymphocytes (CD-4)	T lymphocytes (CD-3)	B lymphocytes (CD-20)
Genotype (I/I)	Genotype (D/I)	Genotype (D/D)
Stable generalized periodontitis	Conditionally stable generalized periodontitis	Progressive generalized periodontitis

Conclusions

The results of correlation analysis indicate that genotype (Del/Del), as polymorphic variant of gene transcription factor NF-κB1, was significantly associated with the emergence of rapidly progressive periodontitis in young people.

Thus, nowadays, there is need to consider in the diagnosis of generalized periodontitis a polymorphic variants of genes, i.e. polymorphism transcription factor NF-κB1, which can enable detailing pathogenetic mechanisms of periodontitis in order to predict the emergence and development of nosology units and might be a genetic background for the justification and development of prevention and treatment measures.

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THE EFFECTIVNESS OF CHRONIC GINGIVITES TREATMENT IN PATIENTS WITH NON-REMOVABLE ORTHODONTIC APPARATUS ACCORDING TO THE RESULTS OF PERIODONTAL TISSUES INDEX ASSESSMENT

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Background. *The risk of chronic gingivitis is increased in patients who undergo orthodontic treatment. It is known that the gingivitis is closely correlated with the level of hygiene.*

Objective. *The study involved 123 orthodontic patients with chronic catarrhal and hypertrophic gingivitis that developed during the first two months of active orthodontic treatment. We chose VITIS ORTHODONTIC (DENTAID INTERNATIONAL, Spain) that contains active ingredients we need to support healthy state of oral cavity. All studied patients were 12–15 years old.*

Methods. *For dental examination we used health indices OHI-S according Greene-Vermillion (1964). Gums bleeding was determined according to a modified SBI index by Muhlemann (1971), inflammation of the gingival margin was assessed by PMA index Parma (1960).*

Results. *Therapeutic treatment consisted of the following: all patients underwent correction of oral hygiene, removal of dental plaque. Vitis ORTHODONTIC was prescribed due to the manufacturer's recommendations: rinse 15 ml for 30 seconds after normal procedures of oral hygiene. Eating or drinking is not recommended during the next 30 minutes after using this product. The results proved a high anti-inflammatory efficacy of the treatment schemes.*

Conclusions. *The complex therapy of early manifestations of inflammation in periodontal had a positive effect on the subjective feelings of patients and health performance rate, gum inflammation and bleeding.*

KEY WORDS: **orthodontic patients; chronic gingivitis; dental examination; oral hygiene.**

Introduction

Dentition abnormalities impair the hygienic condition of the mouth, cariogenic effect exacerbate the situation and increase the risk factors of periodontal diseases. Several researchers indicate high percentage of periodontal abnormalities with dentoalveolar lesions [4, 6, 9, 10]. Thus, the prevalence of periodontal diseases in patients requiring orthodontic treatment was 81.4%. Due to the Anne-Marie Bollen symptoms of periodontal they were determined in 89.3% patients. Periodontal tissues were affected in all kinds of bite anomalies [1].

Thus gingivitis is in deep (46.3%) and an open (43.7%) occlusion, mesial (37.0%) and oblique (33.3%) bite [2].

The clinical picture may correspond to varying degrees of severity of periodontal tissues diseases. Some authors [3, 5] diagnosed chronic periodontitis in 70%, gingivitis – in 30%, including chronic catarrhal gingivitis – in 15%, medium gravity of hypertrophic gingivitis – in 15% of people.

The aim of the study was to improve the efficiency of treatment of catarrhal and hypertrophic gingivitis in patients with fixed orthodontic apparatus in mouth when using rinse aid.

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Methods

The examination and treatment of 123 orthodontic patients in permanent dentition period with symptoms of chronic catarrhal and hypertrophic gingivitis that were determined two months after the beginning of active orthodontic treatment by the technique of permanent braces version ‘the direct arc’. The patients' age was 12–15 years: 59 (47.9%) boys, 64 (52.1%) girls. All patients were almost healthy.

Two weeks before the beginning of orthodontic treatment, all patients were subjected to professional teeth cleaning procedure to remove all deposits and external staining, and

then tooth surface was polished. Before the beginning of the study instructions on oral hygiene were provided for the patients and a standard method of teeth cleaning was recommended.

In a study patients health indices OHI-S according to Greene-Vermillion (1964) were used. Gums bleeding was determined by a modified SBI index by Muhlemann (1971), inflammation of the gingival margin was assessed by PMA index Parma (1960) [7].

The methods of therapeutic treatment consisted of the following: all patients underwent correction of oral hygiene, removal of dental plaque. Vitis ORTHODONTIC was prescribed due to the manufacturer's recommendations: rinse 15 ml for 30 seconds after normal procedures of oral hygiene. Eating or drinking is not recommended during the next 30 minutes after using this product.

In two weeks of using Vitis ORTHODONTIC twice daily, patients were examined again. When assessing the results of the drug, the views of patients about the taste of the drug, convenience of application was surveyed, we also evaluated the dynamics of the major indexes.

Statistical data processing was carried out by probability Student t-test [8].

Results

Initial data of periodontal state in the patients before orthodontic phase of treatment are presented in Table 1. Periodontium was clinically healthy. Hygiene was satisfactory.

Within two months from the beginning of orthodontic treatment, due to the lack of oral hygiene, the availability of items for retention of dental plaque around the brackets, most patients complained of bleeding gums when brushing their teeth, swelling, halitosis.

During objective examination, in 70 patients swelling, cyanosis gum, thickening in the area of gingival papilla were evidenced that pointed to a mild severity of catarrhal gingivitis. Mechanical irritation was accompanied by bleeding. In the teeth there was the increased content of soft plaque (patients avoided brushing due to pain and bleeding gums).

According to the results of objective examination, in 53 children hypertrophic gingivitis was determined that proved the visual assessment of size of crown and vertical sensing. All patients had mild severity of hypertrophic gingivitis that was manifested by proliferation of gum to 1/3 crown. To the touch gingival papillae dense produced false periodontal pockets, bleeding was absent, which evidences of the fibrous form of hypertrophic gingivitis.

For gingivitis diagnosis we used classification of periodontal tissues diseases by MF Danilevsky (1994).

Evaluation of patients' performance in two months after the beginning of orthodontic treatment is presented in Table 2.

All the indicators increased in both groups of patients, oral hygiene index increased to 2.36 points, proving the deterioration in 3 times. The average plaque index increased to 2.6 points that verified unsatisfactory oral hygiene, gingivitis index increased on average by 52%, bleeding index – by 45%.

After the application of hygiene measures and the Vitis ORTHODONTIC solution the patients in 2–3-day reported about reduction of bleeding, gums swelling, disappearance of discomfort events. On examination, the de-

Table 1. Initial data evaluation of the studied patients (M±m)

Indicators	Girls (n=64)	Boys (n=59)
OHI-S (points)	1.00±0.04; p<0.01	1.10±0.03; p<0.01
PMA (%)	18.3±2.8; p<0.05	9.9±1.2; p<0.05
SBI (%)	7.70±0.02; p<0.01	8.50±0.12; p<0.01

Note. Statistical significance of differences between the relevant groups of girls and boys.

Table 2. Evaluation of the patients examination in two months after the beginning of orthodontic treatment (M±m)

Indicators	Chronic catarrhal gingivitis, mild severity (n=70)	Chronic hypertrophic gingivitis, mild severity, fibrous form (n=53)
OHI-S (points)	2.21±0.04; p_1<0.05	2.51±0.03; p_1<0.05
PMA (%)	69.35±4.30; p_1<0.05	71.67±3.70; p_1<0.05
SBI (%)	47.17±0.14; p_1<0.05	57.16±0.10; p_1<0.01

Note. p_1 – difference reliability index compared with the initial data.

crease of gingival hyperemia was determined. In 8–10 days of the treatment, hyperemia, gingival swelling disappeared, gingival papillae compacted and acquired normal form. By the 14th day of rinses the state of oral health improved significantly. Also with a significant improvement of subjective sensations the patients noted positive dynamics of the indices, and there was no oral mucosa irritant. None of them had any allergic reaction or any adverse side effects. All patients had good rinse results. Teeth sensitivity was not changed. Teeth were not stained. The results are presented in Table 3.

Table 3. Evaluation of patients examination in two weeks after the treatment (M±m)

Indicators	Chronic catarrhal gingivitis (n=70)	Chronic hypertrophic gingivitis (n=53)
OHI-S (points)	0.80±0.04; p2<0.05	0.90±0.03; p2<0.01
PMA (%)	16.3±2.8; p2<0.05	19.9±1.7; p2<0.05
SBI (%)	8.20±0.12; p2<0.05	10.50±0.04; p2<0.05

Note. p_2 – differences reliability index compared with the initial data in two months after the beginning of orthodontic treatment.

Discussion

The results of epidemiological studies of dental status of children in some areas of Ukraine prove great variability and frequency of teeth anomalies, which varies in different regions from 30.8% to 85.4% and tends to increase. The treatment of anomalies of jaw apparatus teeth using fixed orthodontic structures is a priority in contemporary orthodontics, because it is highly effective and provides reliable retention of the obtained results. However, in the literature it is mentioned that periodontal tissue react to the treatment with braces, whereby the share of gingivitis according to Petrushanko TA (2013) is 33.3% [3,4,5]. Among the causes of inflammatory diseases of periodontal tissues in children this categories are defined: worsening of hygienic condition of oral cavity, microbial factors, hormonal changes and the effect of orthodontic forces. However, the development of complex therapeutic measures for chronic catarrhal and hypertrophic gingivitis in children with non-removable orthodontic apparatus has its own significance and importance that should be considered.

Thus, a comparative analysis of clinical trials revealed that the developed complex of therapeutic measures for patients with braces help to improve its clinical course that made it possible to achieve stable remission.

Conclusions

Inclusion in the complex therapy of early manifestations of inflammation in periodontium had a positive effect on the subjective feelings of patients and health performance rate, gum inflammation and bleeding. It is necessary to emphasize that the use of Vitis ORTHODONTIC must be preceded by correction of oral hygiene and improvement of hygiene practices in patients.

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INFLUENCE OF *LACTOBACILLUS* SPP. ON COLONIZATION AND ANTI-INFECTIOUS RESISTANCE OF THE MUCOUS MEMBRANES OF THE UPPER RESPIRATORY TRACT

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Background. *Lactobacilli are very important for the formation of colonization resistance and have pronounced antagonistic effect against a wide range of microorganisms. That is why the lactobacilli have extensive use as a component of classic probiotic agents that are widely used to prevent and treat dysbiotic conditions of digestive and genital systems of people.*

Objective. *The aim of the research was to study the effect of lactobacilli on anti-infectious resistance of mucous membranes of upper respiratory tract.*

Methods. *The colonization degree (lg CFU / g) of nasal mucosal membranes by Lactobacillus spp. and S. aureus was determined in all carriers before the experiment. Also, the level of lysozyme and secretory immunoglobulin A (sIgA) in nasal secretions cavities was identified.*

Results. *It was established a clear dysfunction of anti-infectious resistance in carriers of Staphylococcus aureus - a decrease of colonization resistance and local immunity of mucous membranes of upper respiratory tract. As for the anti-infectious resistance of nasal mucosal of S. aureus carriers, the level of lysozyme and secretory immunoglobulin A gradually increased after the application of probiotic strain L. rhamnosus GG, and in 21 days it reached rates of healthy individuals.*

Conclusions. *It was found out that probiotics for nasal passages sanitation in Staphylococcus aureus carriers lead to gradual eradication of the pathogen (S. aureus) with restoration of colonization and anti-infectious resistance, mucous membranes and upper respiratory tract.*

KEYWORDS: ***Lactobacillus* spp.; lysozyme; sIgA; upper respiratory tract.**

Introduction

Lactobacilli are widely distributed in the environment and have a high biological activity. They are an important part of normal microflora of digestive and genital human tracts and they belong to the resident microflora of nasopharynx [1]. Lactobacilli are actively involved in the formation of colonization resistance of mucous membranes and have significant antagonistic action against a wide range of bacteria [2-4]. That is why the lactobacilli are widely used as a component of classic probiotic agents [3-5]. Probiotics based on lactobacilli are widely used to prevent and treat dysbiotic conditions of digestive and genital human system [6-8]. Nowadays, research on the use of lactobacilli for prevention or treatment of infections and recovery of anti-infectious resistance of mucous membranes of upper respiratory tract (URT) are actual [9].

The aim of the research was to study the effect of *Lactobacillus* spp. on colonization and anti-infectious resistance of mucosal membranes of upper respiratory tract in *Staphylococcus aureus* carriers. The objects of the study were the carriers of Staphylococcus aureus (n=29) among the medical staff of a hospital, Kharkiv (Ukraine).

Methods

The colonization degree (lg CFU / g) of nasal mucosal membranes by *Lactobacillus spp*. and *S. aureus* was determined in all the carriers before the experiment. Also, the level of lysozyme and secretory immunoglobulin A

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(sIgA) in nasal secretions cavities was identified. Then the carriers were divided into groups: I (n=7, control) – the persons, who received saline as the sanitation agent (0.85% NaCl, pH 7.2) during 30 days, 2–3 drops twice per day in each nostril; II (n=11) – the persons, who received as the sanitation agent a suspension of probiotic strain *L. rhamnosus* GG (in saline) at a concentration of 5×10^9 CFU/ml (according to the group I scheme). The degree of colonization of nasal mucous membranes by *Lactobacillus spp.* and *S. aure*us, lysozyme and sIgA levels in nasal cavities secretions were determined in the individuals taking part in the experiments repeatedly in 7, 14 and 21 days of sanitation. The material was selected as follows: in each nostril, alternatively, with special replicator sterile foam tape was injected and mucus impregnation was held for 5 minutes, after it the tape was immersed in a sterile tube containing 1.0 ml of phosphate-buffered saline (PBS, pH 7.2) for 30 minutes and then separated from the foam carrier mucus by insulin syringe, forcing it into the PBS.

In the comparisons group there were persons, who did not have upper respiratory tract infection within 6 months (almost healthy, n=15).

Lysozyme levels were determined by means of nephelometric method [10]. The concentration of lysozyme was rated in micrograms/ml (μg/ml). To determine sIgA, RIDASCREEN®sIgA ELISA kits of R-Biopharm AG Company (Germany) were used. The results of calculations were carried out on Stat Fax enzyme immunoassay analyser. sIgA concentration was rated in mg/ml.

Results

It was established that in the healthy individuals (n=15) the degree of colonization of the nasal mucous membranes *Lactobacillus* spp. was 6.64±0.9 lg CFU/g, the amount of lysozyme and sIgA in nasal secretions cavities were respectively at 17.45±2.9 μg/ml and 129.3±21.7 mg/ml.

The degree of colonization of nasal mucosa Lactobacillus spp. was 2.2±0.7 lg CFU/g, the amount of lysozyme and sIgA in nasal secretions cavities were respectively 7.45±2.1 μg/ml and 63.9±19.8 mg/ml in the *S. aureus* carriers.

Discussion

Consequently, the degree of colonization of nasal mucosa Lactobacillus spp. in the carriers of Staphylococcus aureus was in 3.8–5.0 ($p<0.01$) times lower compared to the healthy individuals. The amount of lysozyme and sIgA in nasal secretions cavities were also lower in comparison with the healthy individuals, respectively in 1.5–3.8 ($p<0.05$), and 1.2–3.4 ($p<0.05$) times.

Thus, it was established a clear dysfunction of anti-infectious resistance in the carriers of *Staphylococcus aureus:* the decrease of colonization resistance and local immunity of mucous membranes of upper respiratory tract.

According to the literature, application of probiotics or synbiotic treatment regimens in respiratory infections reduces relapse respiratory tract infections and bronchial obstruction in children [9]. But the authors did not conduct any studies to determine the number of lactobacilli in secrets of URT and their influence on anti-infectious resistance of mucous membranes. In connection with the foregoing, we conducted a study on the possibility of recovery colonization and anti-infectious resistance of mucous membranes of upper respiratory tract in the carriers of *S. aureus* using the probiotic strain *L. rhamnosus* GG.

The data in the Table 1 prove that the probiotic strain *L. rhamnosus* GG for sanitation the carriers decreased the degree of colonization by Staphylococcus aureus mucosal in 7 and 14 days after the sanitation of lactobacilli suspension, and in 21 days there was a complete eradication of *S. aureus* from nasal mucous membranes. Moreover, the decrease of *S. aureus* degree colonization on mucosal of the carriers occurred against the background of increase in the number of *Lactobacillus spp*. on mucous membranes.

As for the anti-infectious resistance of nasal mucosal of *S. aureus* carriers, the level of lysozyme and secretory immunoglobulin A after the application of the probiotic strain *L. rham-*

Table 1. Average indexes of mucosal colonization resistance after sanitation, (M±m)

Groups	The degree of mucosal colonization URT, lg CFU / g					
	Lactobacillus spp.			*S. aureus*		
	in 7 days	in 14 days	in 21 days	in 7 days	in 14 days	in 21 days
I	2.3±0.7	2.2±0.6	2.1±0.7	4.7±0.7	4.9±0.6	4.8±0.6
II	3.8±0.4*	4.5±0.4*	5.3±0.4*	1.8±0.6*	0.6±0.6*	0±0*

Note: * – significant difference between these indexes of I and II groups ($p<0.05$).

Table 2. Average indexes of mucosal anti-infectious resistance after sanitation, (M±m)

Group	Indicator of local immunity					
	level of lysozyme, μg/ml			level of sIgA, mg/ml		
	in 7 days	in 14 days	in 21 days	in 7 days	in 14 days	in 21 days
I	7.3±2.4	7.4±2.2	7.4±2.3	61.3±21.3	62.1±18.8	61.9±19.3
II	11.6±3.3*	13.3±2.9*	15.4±2.7*	84.8±19.8*	102.9±21.1*	112.2±19.1*

Note: * – significant difference between these indexes of I and II groups ($p<0.05$).

nosus GG significantly increased, and in 21 days it reached rates of healthy individuals (Table 2).

To be precise, after the sanitation of probiotic nasal of the carriers Staphylococcus aureus, the level of lysozyme and sIgA rose: 7 days, an average of 1.6 ($p<0.05$) and 1.4 ($p<0.05$) times, respectively; at 14 days of 1.8 ($p<0.05$) and 1.6 ($p<0.05$) times, respectively; after 21 days – 2.1 ($p<0.05$) and 1.8 ($p<0.05$) times, respectively, compared with the group the carriers, that sanitation was conducted with saline.

Conclusions

Thus, it was found that the application of probiotics for nasal passages sanitation in the carriers of *S. aureus* leads to the gradual eradication of the pathogen (*S. aureus*) with colonization restoration (increased degree of mucosal lactobacilli settlement), and anti-infectious resistance (increased level of sIgA and lysozyme) in mucous membranes and upper respiratory tract.

This study demonstrated the positive effect of *Lactobacillus spp*. on the formation of colonization and anti-infectious resistance of upper respiratory tract mucous membranes and provided an opportunity to consider probiotics as a useful option for immune exposure to chronic infections of staphylococcal origin.

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OPERATIONS THROUGHPUT AS A DETERMINANT OF GOLDEN-HOUR IN MASS-GATHERING MEDICINE

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Background. *Golden-hour, a time-tested concept for trauma-care, involves a systems approach encompassing healthcare, logistics, geographical, environmental and temporal variables. Golden-hour paradigm in mass-gathering-medicine such as the Hajj-pilgrimage entwines along healthcare availability, accessibility, efficiency and interoperability; expanding from the patient-centric to public-health centric approach. The realm of mass-gathering-medicine invokes an opportunity for incorporating operations-throughput as a determinant of golden-hour for overall capacity-building and interoperability.*

Methods. *Golden-hour was evaluated during the Indian-Medical-Mission operations for Hajj-2016; which established, operated and coordinated a strategic network of round-the-clock medical operations. Throughput was evaluated as deliverables/time, against established Standard-Operating-Procedures for various clinical, investigation, drug-dispensing and patient-transfer algorithms. Patient encounter-time, waiting-time, turnaround-time were assessed throughout echeloned healthcare under a patient-centric healthcare-delivery model. Dynamic evaluation was carried out to cater for variation and heterogeneity.*

Results. *Massive surge of 394 013 patients comprising 225 103 males (57.1%) and 168 910 females (42.9%) overwhelmed the throughput capacities of outpatient attendance, pharmacy, laboratory, imaging, ambulance, referrals and documentation. There was a delay in attendance, suspicion, diagnosis and isolation of patients with communicable infections. The situational-analysis of operations-throughput highlights wasted turnaround-time due to mobilization of medical-team, diverting critical healthcare resources away from emergency situations.*

Conclusions. *Time being a crucial factor in the complexity of medical-care, operations-throughput remains an important determinant towards interoperability of bottlenecks, thereby being a determinant of golden-hour in mass-gathering-medicine. Early transportation of a patient to definitive-care reduces treatment initiation-time, notwithstanding logistics of communication, evacuation, terrain and weather being deterministic in outcome. Golden-hour needs to be emphasized under a population-based approach targeting the clientele towards administering first-aid and reaching out to hospital within the golden-hour.*

KEY WORDS: **golden-hour; operations throughput; mass-gathering medicine; turnaround-time; definitive-care; population-based approach.**

Introduction

Golden-hour, a hitherto time-tested concept for trauma-care, has been found useful across the entire ambit of emergency health-systems. Golden-hour involves a systems approach encompassing healthcare, logistics, geographical, environmental and temporal variables.

Mass-Gathering-Medicine applies to situations where a mass-gathering overwhelms accessibility, interoperability and public-safety response to medical-emergencies. Mass-Gathering-Medicine involves higher rates of morbidity and mortality attributable to infections, trauma, environment, occupation, lifestyle, substance-abuse and disasters [1, 2]. Hajj pilgrimage is a 5-day outdoor unbounded peaceful mass-gathering involving a moving assemblage of over 3.5 million pilgrims from 200 countries, engaged in prayers, supplications and strenuous rituals in densities of 9 people/m^2 or more, in harsh desert climate of Saudi-Arabia. Mass-Gathering-Medicine at Hajj is challenged by issues of healthcare availability, accessibility, infection control, rapid-diagnosis, on-site treatment, referral, evacuation, and response to disasters and public-health emergencies [3, 4].

The realm of Mass-Gathering-Medicine invokes an opportunity for incorporating operations-throughput as a determinant of golden-

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hour for overall capacity-building and interoperability. This prospective study explored the perspective of golden-hour across the spectrum of Mass-Gathering-Medicine.

Methods

The perspective of golden-hour as a determinant of operations-throughput was evaluated during the Indian Medical Mission operations for Hajj-2016, which established, operated and coordinated a strategic network of round-the-clock medical operations in Mecca, Medina and Jeddah during Hajj-2016 pilgrimage from 01 Aug to 30 Oct 2016. The mission framework comprised of (a) Mobile medical task-forces detailed for strategic mass-gathering locations such as mosques, religious places, bus-stops, train-stations and along the pilgrimage assemblage during the five critical days of Hajj, to cater for 5000-100000 pilgrims per congregation. (b) 18 Static-clinics having basic first-aid capabilities catered for 6000-9000 pilgrims/clinic residing in over 400 buildings in Mecca and Medina (c) One mobile referral tent-clinic with 35 tent-clinics having medical attendance facilities only, catered for 3000-4000 pilgrims in over 5000 tents in Mina and Arafat, as well as 1,40,000 unsheltered pilgrims in Muzdalifah. (d) Two strategically sited 40-bedded secondary-care referral facilities for critical-care, internal-medicine, general and orthopaedic surgery, gynaecology, paediatrics, psychiatry, dermatology, isolation, lab-medicine and radiology (e) Tertiary-care patient transfers coordinated with 28 Saudi Arabian hospitals.

Golden-hour as a determinant of operations throughput was evaluated across the spectrum of Mass-Gathering-Medicine, through evaluation of throughput in terms of deliverables/per unit time, against established Standard-Operating-Procedures for various clinical, investigation, drug-dispensing, patient-transfer, administrative and patient-safety algorithms.

The average range of patient encounter-time, waiting-time, turnaround-time were assessed throughout various echelons of healthcare under a patient-centric healthcare delivery model. Even low-acuity patients were attended round-the-clock. Subjective assessment of communication-time, visit-time and doctor-patient interaction on-site was done. Encounter-time was defined as time taken by the patient for a healthcare operation such as outpatient attendance, investigation, drug-dispensing, minor-procedures and ambulance transfers. Waiting-time was defined as pre-operation waiting time due to resource limitations attributable to health-system, while excluding waiting-time attributable to patient or otherwise such as delayed reporting, traffic-conditions and post-attendance time for decisions or other personal reasons. Turnaround-time was defined as time taken to complete a task incorporating both operation and waiting-time, as applicable. Communication-time/reporting-time/accessibility-time or patient-rescue time was defined as time taken for health-intelligence to reach the doctor. Visit-time was defined as time taken for visit by medical team to reach the patient through ambulance/foot. Doctor-patient interaction on-site was defined as time taken for elicitation of relevant history and administration of first-aid, if any. The turnaround-time for healthcare personnel was compared between site visits and that with patient being brought to healthcare facility. Health emergency was defined as any condition threatening the life or limb of a patient. Initiation of definitive-care was the end/start point of defining upstream (pre-hospital) and downstream (hospital-care) processes, being prior and after respectively. Dynamic evaluation was carried out to cater for variation and heterogeneity.

Results

A total of 394 013 patients comprising 225 103 males (57.1%), and 168 910 females (42.9%) were attended by a team of 144 doctors including 50 specialists, 146 paramedics and 74 ancillary staff. The patient distribution in mobile medical task-forces, static-clinics and tent-clinics was 13473, 374475 and 5135 patients. Out of 930 secondary-care and 523 tertiary-care referrals, 585 and 495 patients were institutionalized respectively. Total secondary-care bed days were 4626, average bed occupancy being 77.78% for one month around Hajj and 32% otherwise. Pooled unadjusted average length of stay of all patients was three days. 1505 minor surgical and 770 orthopaedic procedures were performed. 7850 laboratory, 2074 imaging and 1159 electrocardiograms were carried out.

Massive surge of patients overwhelmed the throughput capacities of outpatient attendance, pharmacy, laboratory, imaging, ambulance, referrals and documentation. There was delay in attendance, suspicion, diagnosis and isolation of patients with communicable infections. Average encounter-time, waiting-time and operation turnaround-time for patients,

procedures, investigations and ambulance operations is depicted in Table 1. The turnaround-time for healthcare personnel has been compared in Table 2.

Discussion

Golden-hour

Golden-hour conceptualizes early arrival of patient to definitive-care enabling early recog-

Table 1. Indian Medical Mission for Hajj–2016: encounter, waiting and operation turnaround time for patients, procedures, investigations and ambulance operations

No.	Patient–care variables	Encounter time (minutes)	Waiting time (minutes)	Operation turnaround time (minutes)
Pre–Hospital patient care (on-site primary-care through mobile-medical-task-force during mass–gathering congregations)				
1	Rescue/reporting/accessibility time to healthcare professional	1–30	0–5	20–60
2	Immediate first-aid	5–20	0–5	20–60
3	Ambulance arrival at site of injury/first-aid	0–15	0–15	30–150
4	Stretcher/wheelchair transfer from site of first-aid to ambulance/primary-care/secondary-care	0–20	0–2	30–150
5	Ambulance transfer from site of first-aid to primary/secondary-care	10–40	10–20	30–150
Pre-Hospital patient care (static-clinic based primary-care)				
1	Low acuity patients	2–10	0–60	2–60
2	High acuity patients	20–40	0–5	10–60
3	Checking of vitals	3–5	0–30	3–30
4	Systemic examination	2–15	0–15	2–15
5	Blood glucose by glucometer	2–3	0–40	2–40
6	Collection of medicines from pharmacy	2–10	0–60	2–60
7	Ambulance transfer from static-clinic to secondary/tertiary-care	30–90	0–60	30–150
Pre-Hospital patient care (tent-clinic based primary-care during five critical days of Hajj)				
1	Low acuity patients	2–10	0–20	2–20
2	High acuity patients	20–40	0–2	20–40
3	Checking of vitals	3–5	0–10	3–10
4	Systemic examination	2–15	0–10	2–25
5	Blood glucose by glucometer	2–3	0–10	2–10
6	Collection of medicines from pharmacy	2–10	0–20	2–20
7	Ambulance transfer from tent-clinic to secondary/tertiary-care	10–20	0–30	10–60
Hospital-based patient care (secondary-care)				
1	Low acuity patients	2–5	0–60	2–60
2	High acuity patients	20–40	0–2	20–40
3	Electrocardiogram	5	0–30	5–30
4	Urine routine and microscopy	10	0–20	10–20
5	Blood glucose by glucometer	2–3	0–40	2–40
6	Malaria/ Dengue/HCV by rapid kits	30	0–30	30–60
7	Haematology tests	30	0–30	30–60
8	Clinical chemistry tests	30	0–30	30–60
9	Manual X-ray	20	0–30	30–60
10	Ultrasonography	5	0–20	5–20
11	Minor surgical procedures	30	0–60	30–60
12	Orthopaedic procedures	30	0–60	30–60
13	Collection of medicines from pharmacy	2–10	0–60	2–60
14	Ambulance transfer from secondary-care to tertiary-care	30–60	0–60	60–180

Table 2. Comparative analysis of average time of initiation of definitive-care

No.	Healthcare personnel turnaround time	Site/home visit by doctor	Patient brought to healthcare facility
1	Mobile-medical-task-force during Mass-gathering congregations	20–60	5–30
2	Tent-clinic based primary-care	10–30	5–15
3	Static-clinic based primary-care	10–60	5–15
4	Hospital-based secondary-care	10–60	5–30
5	Tertiary-care		

nition of disease-condition, triage, initiation of resuscitation, control of bleeding, intravenous fluids, antimicrobials, analgesics, investigations and treatment/surgery, thereby positioning the patient on the path to recovery [5]. The model has evolved with expansion in scope from trauma to head-injury, sepsis, shock, stroke, neonates, antimicrobial therapy, pressure-ulcer; weaning of mechanical resuscitation, ICU admissions, ischemia, heat-stroke, laboratory, imaging, referral and evacuation etc. as well as expansion in time to evolve the "platinum half-hour", "golden 10 minutes" and "silver-day" [6-9].

Golden-hour is an essential pragmatic tool in pre-hospital care in both clinical and logistic fronts. Golden-hour subsumes time to reach definitive-care, thereby including communication/accessibility/reporting time, doctor-patient interaction time and stabilization-time allowing critical clinical interventions. Significant associations with deterioration of patient's condition have been found with each incremental minute of patient arrival to definitive-care [6-9]. It is important to note that doctor-patient interaction time and stabilization-time may be as long as 27.55 minutes, which may nudge into the golden-hour [10]. Logistics of communication, evacuation, terrain and weather considerations are deterministic in outcome [11-15].

There are three axes to golden-hour. Firstly, golden-hour in a patient-centric scenario incorporates resource maximization towards protocolized treatment. Secondly, golden-hour in an incident-accident centric scenario involves triage and evacuation under the 'best for most' approach. Thirdly, golden-hour in mass-gathering-medicine insinuates a resource-limited scenario with surge of both high-acuity and low-acuity patient-crowds from diverse socio-cultural backgrounds, expectations and needs [16]. The plot of golden-hour in mass-gathering-medicine is shown in Fig. 1.

While patients' arrival within first 60 minutes has been associated with better prognosis and early discharge, increased out-of-hospital time is associated with the contrary. Pre-hospital life-saving procedures such as intubation may not reduce morbidity [17-18]. Communication of information is critical for preparedness. Even when encounter-time and waiting-time may be few minutes, together they lead to a turnaround-time spilling out of the golden-hour. Ambulance turnaround-time are affected by confusing addresses of camps/sites, distance, overwhelming vehicle and pedestrian traffic as well as reorganized routes, which may lead to unusual delay. Huge surge of patients lead to crowding of healthcare facilities reduces access to more deserving patients. Little understanding of health emergencies by patients lead to increased waiting-time for patients deserving early attention [6, 19].

Throughput in healthcare

Human system is an extremely complex system where existing knowledge is complemented by deductive algorithm and modelling to design protocolized goal-directed diagnosis and treatment modules. The throughput involving human lives is dependent on doctor-patient interactions, which incorporates real-life human dependence at both ends; and poses limitations in simulation-based real-time preparedness. Despite advances in biomedical engineering, the throughput in health-systems

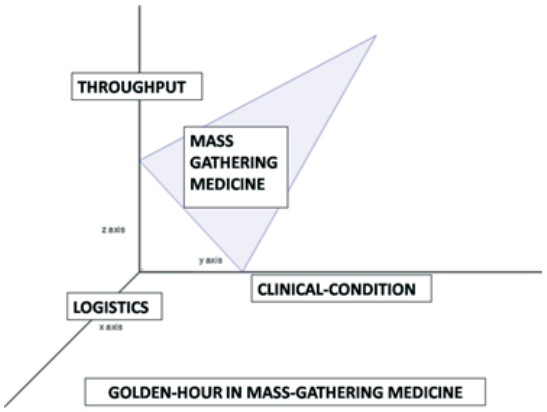

Fig. 1. Illustration of Golden-Hour in Mass-Gathering Medicine.

is not comparable with inanimate entities such as data packets, industrial processes and products; which function in systems invented, designed, standardized and calibrated through calculations and simulations. The throughput in medicine and health systems has had no defined maximum since antiquity, even in the absence of stochastic and deterministic deductions and quantifiable indicators. Disasters, wars and pandemics have exemplified the doctor-patient equation in situations of overwhelming throughput. The concept of maximum theoretical throughput may at best be applied to quality healthcare including documentation which is restricted in scope in an overwhelming throughput scenario. The maximum achievable throughput is defined by resource-limitations [20, 21].

Golden-hour in mass-gathering medicine

Golden-hour paradigm in mass-gathering medicine entwines along healthcare availability, accessibility, efficiency and interoperability; expanding from the patient-centric to public-health centric approach. Operational stringencies exist parallel to infrastructural and provisioning stringencies in makeshift healthcare facilities with intricate operational, procurement, distribution and disposal logistics. Overwhelming number of patients may lead to downgrading of medical centres to resource-limited setups, compromising standards of healthcare, clientele satisfaction and resource security. [22] It also leads to physical, mental and compassion fatigue amongst healthcare-personnel. Healthcare availability involves dynamic planning of human, auxiliary and ancillary resources such as infrastructure, medicines, equipment and consumables. Human resources are a critical component of golden-hour mandating deployment discretion. Healthcare professionals are often required to multi-task in resource-limited settings which hamper the technical efficiency towards focused and dedicated clinical work. Accessibility can be improved by resource planning, information technology, communications, surveillance systems and outreach activities. Internal communication for healthcare professionals should include information on local diseases, transmission, antimicrobial resistance, availability of medicines through acquired health intelligence [20, 21].

Population-based approach to Golden-hour

The role of population is often not discussed within the Golden-hour concept. Golden-hour pre-hospital care is ought to be provided by people such as family, friends and the first people noticing the need; much before the arrival of first responders, paramedics or doctors. While significant expertise in first-aid may exist in progressive Western communities, negligible help is available to victims in most developing communities leading to increased morbidity and mortality. The problem of spectator-inertia around accidents mandates behavioural change in attitude of the masses towards philanthropic efforts. Community level expertise development programs can enable rapid first-aid availability. Calling for a paramedic/doctor to reach the site is time consuming and doesn't lead to definitive-treatment as most cases need to be shifted to hospitals for simple measures such as fluid resuscitation, application of plaster-casts or administration of analgesics, antiallergics etc. Instead of sending a representative from the community to call for medical help, the time and effort should be utilized towards providing initial care and transportation support to the victim towards the hospital for definitive-care. Patient being brought to definitive-care reduces treatment initiation-time by 50% as seen in this study. While the patient is brought early to a resource-rich environment compared to pre-hospital care, it also furthers simultaneous domiciliary care and monitoring of multiple patients. A multifold availability of doctor and auxiliary health professionals at medical facility facilitates adherence to protocols and optimization of processes, thereby improving outcome and streamlining throughput [19, 23, 24].

The situational-analysis of operations throughput highlights wastage of considerable time due to mobilization of medical team from the hospital to reach the patient on-site due to calls for low/moderate-acuity complaints resulting from inability of the patients to judge medical emergencies. It has been proven that trauma team activation doesn't guarantee better survival [19]. Visits consume the turnaround time for healthcare professionals, thereby reducing their availability to attend to deserving patients in need, and diverted critical healthcare resources away from emergency situations. Sometimes, visit come at the cost of leaving the medical facility without a doctor. In any resource limited scenario, most patients who deserve time, access definitive-care beyond the golden-hour [7, 8, 25, 26].

There is an evolving paradigm of healthcare which is as explicit as right-to-heath or implicit under human/social rights/security within the

boundaries defined by a nation/state for its citizens, or by an employer/insurer for beneficiaries. The kingdom of Saudi Arabia has been magnanimous to extend comprehensive quality healthcare to Hajj pilgrims from all over the world, through a robust and efficient state-sponsored single-tier health system, a vanguard equity healthcare-model difficult to emulate. Nevertheless, operations throughput gets overwhelmed during Hajj at all healthcare facilities [1, 2, 25].

The golden-hour is not a blind concept around buying time; rather it is a dynamic concept requiring reasonable discretion and prudence; failing which there exist limitations to its applicability. Overwhelming fluid resuscitation and extensive procedures may lead to procedural errors, transmission of infections and antimicrobial overuse [17, 27-30]. Since the study catered to 4.5 lakh patients across a wide network of medical facilities in a setting of mass-gathering medicine, limitations exist due to variability in patients, sites and medical facilities. Elements of bias and confounding at patient and doctors' may not be fully accounted. The variability of operations as well as that of observers may limit the exhaustive collation of data.

Conclusions

Time being a crucial factor in the complexity of medical-care, operations throughput remains an important determinant towards availability, accessibility, efficiency and interoperability of bottlenecks, thereby being a determinant of golden-hour in mass-gathering-medicine. Early transportation of patient to definitive-care reduces treatment initiation-time by 50% thereby improving outcome, and consequentially emphasizing patient-evacuations personal visits and interventions by medical/trauma team. Evidence-based guidelines, standards, alert and response systems need to be developed with international cooperation and networking for enhancing capability and core competency. Golden-hour needs to be emphasized under a population-based approach targeting the clientele towards administering first-aid and reaching out to hospital within the golden-hour.

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AWARENESS OF LYME BORRELIOSIS OF THE PATIENTS OF TERNOPIL REGIONAL TB DISPENSARY

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Background. *Lyme disease has many clinical features similar tothose in sarcoidosis and tuberculosis. Epidemiological data in the world, in particular in Ukraine, proves the increase in Lyme borreliosis incidence. Ternopil region is endemic with Lyme borreliosis.*

Objective. *The research was aimed to investigate the prevalence of infection with Borrelia burgdorferi and epidemiology features of borreliosis among the patients of Ternopil Regional TB Dispensary.*

Methods. *In total, 29 patients were admitted to Departments of Differential Diagnostic, TB Therapy and TB Surgery of Ternopil Regional TB Dispensary in October 2016-January 2017. All the surveyed answered the questions of an integrated international questionnaire, where they noted the area and a number of tick bites, described the removal method, noted the survey for borreliosis pathogen and complaints after tick bites.*

Results. *It was established that 5 respondents had a history of tick bites episodes, but only in one case the patient was examined of borreliosis. Tick bites were noticed in 3 patients with sarcoidosis and 1 with tuberculosis (TB) and exudative pleurisy, respectively.*

Conclusions. *The absence of appeals for medical care, lack of sufficient information on Lyme borreliosis and disuse of preventive measures for tick bites by the interviewed patients of Ternopil regional TB dispensary departments proves the need of improvement of health education on Lyme borreliosis (LB) among this category of population. 24 (82.7%) of 29 respondents did not remember the tick bite. The symptoms of (LB) are similar to those in sarcoidosis and tuberculosis (pleural lesions, heart, joints, nervous system, skin), and the presence of tick bites gives the reasons to examine these patients of Borrelia burgdorferi senso lato.*

KEY WORDS: **Lyme borreliosis; survey; sarcoidosis; tuberculosis; granulomatous diseases; Lyme disease; Borrelia burgdorferi.**

Intoduction

Lyme disease (systemic tick borreliosis, Lyme borreliosis, chronic migrating erythema, clip erythema) is defined as the natural cell transmissible disease that is caused by Borrelia burgdorferi and is manifested with erythema migrans, fever, lesions of the central and peripheral nervous systems, heart and large joints [1, 3] Spirochetes are carried by Ixodes tick. The epidemiological features of LB in the world, in particular in Ukraine, proved the annual incidence increase [5, 12, 15, 17]. Due to the location in the zone of moderate continental climate with warm humid summers and mild winters, chernozem, broadleaf and mixed forests, the territory of Ternopil region is endemic with LB.

Spring-summer seasonality is usual for tick bites. But in recent years for Ixodes ticks winter diapause takes place earlier, and seasonal activity continues till the third week of November, which leads to an increase in the number of ticks and, consequently, a large number of attacks on people. In 2016 the first tick bite in Ternopil happened on the third week of February. During 10 months of 2016 1,211 cases of tick bites (in all districts of Ternopil region) were compared to 1289 in the same period of 2015. According to the primary urgent messages in 2016 135 cases of Lyme disease were registered compared to 100 in the same period in 2015 [11].

According to the literature, Borrelia burgdorferi can act as a potential cause of systemic granulomatous disease such as sarcoidosis [2,

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4, 13, 14]. However, sarcoidosis can be caused by other etiological factors: infections (viruses: herpes, Epstein-Barr, retrovirus, coxsackie B virus, cytomegalovirus, bacteria: Propionibacterium acnes, Mycobacterium tuberculosis and other mycobacteria, Mycoplasma), inorganic (beryllium, zirconium and aluminum) and organic (pollen, clay) agents [2, 4, 7, 10, 16]. Lyme disease has many symptoms similar to those in sarcoidosis and tuberculosis (lesions of pleura, heart, joints, nervous system, skin) [6, 8, 9, 18]. Assumptions about of borreliosis and sarcoidosis connection are based on the geographical spread of both diseases and family manifestations of these diseases [2, 4, 13]. Therefore, the accuracy of sarcoidosis diagnosis depends on the thorough surveys of the patients with potential contact to organic and inorganic antigens [2, 10].

The objective of the study was to investigate the prevalence of Borrelia burgdorferi infection and epidemiological features of borreliosis among the patients of Ternopil Regional TB Dispensary. The survey is a part of a joint Ukrainian-Polish project "Research on the epidemiology, pathogenesis, clinical manifestations and prophylaxis of borreliosis" together with the Pope John Paul II State School of Higher Education in Biala Podlaska (Poland) within the research projects of the European Union.

Methods

In total, 29 patients were admitted to the Departments of Differential Diagnostic, TB Therapy and TB Surgery of Ternopil Regional TB Dispensary in October 2016-January 2017. The age of the respondents ranged from 20 to 65 years old. There were 17 (58.6%) men, 12 (41.4%) women. All the surveyed answered the questions of an integrated international questionnaire, where they noted the area and a number of tick bites, described the removal method, noted the survey for borreliosis pathogen and complaints after tick bites. The patients of Ternopil Regional TB Dispensary departments also informed about the use of repellent at the entrance to the forest zone and the checking the skin after leaving it. The analysis of patients' awareness and media data about borreliosis, according to the questionnaires, proved the need of enhancement of their knowledge of Lyme disease or other diseases associated with tick bites.

Results

Among the respondents city residents prevailed: 18 men (62.1%), 11 (37.9%) rural residents. Due to the nosology there were 17 (58.6%) of the patients submitted with sarcoidosis, 5 (17.2%) with pulmonary tuberculosis and 7 (24.1%) with pulmonary tuberculosis complicated with exudative pleurisy. 23 (79.3%) of the respondents suffered from chronic diseases.

Among the surveyed 29 patients 5 (17.3%) of the respondents noted the tick bites, 24 (82.7%) did not remember the episode of bite. One respondent noticed the appearance of the erythema on the site of the bite within 12 hours.

5 respondents described the localization of tick bites. It should be noted that the most common tick bites the patients noticed on the trunk (2) and lower limbs (2). Only one person noticed tick bites on the abdomen. Most of the bites took place in the forest – 4, in rural areas – 1.

All the patients removed the tick be themselves (with fingers) within to 12 hours after the bite. The patients with erythema migrans or other symptoms did not ask for medical aid. According to the survey, only one respondent was examined to borreliosis. The diagnosis was confirmed by western blot and the patient was treated with macrolide antibiotics.

According to the results of the survey the awareness of borreliosis in patients of Ternopil Regional TB Dispensary is insufficient.

Table 1. Characteristic features of the patients

Nosology	Number of patients	Number of patients %	Information about episode of tick bite	Information about the mites (patients) surveyed on Lyme borreliosis
Sarcoidosis	17	58.6	3	1
Pulmonary tuberculosis	5	17.2	1	–
Pulmonary tuberculosis complicated with exudative pleurisy	7	24.2	1	–
Total	29	100	5	1

Table 2. Multiplicity of tick bites

Multiplicity of tick bites	Number of tick bites (n=5)	Number of tick bites, %
1 time	2	40
2 times	1	20
many (3 and more times)	2	40

Table 3. Localization of tick bites

Localization of bite	Number of bites (n=5)	Number of bites,%
hands	–	–
legs	2	40
trunk front back	2	40
abdomen	1	20
neck	–	

Quality medical history of illness and examination of patients is important for differential diagnosis of granulomatosis and LB for timely adequate therapy. Incorrect or late diagnosis of LB may cause the development of complications in patients [3, 5, 15].

Discussion

According to the literature, tuberculosis and sarcoidosis histologically belongs to granulomatous diseases, which are based on caseous and non caseous granuloma. While tuberculosis agent is verified, sarcoidosis is a systemic disease which aetiology is still unknown [2]. However, according to the literature, the probable causes of sarcoidosis may be Mycobacterium tuberculosis, Borrelia burgdorferi, Propiono bacterium acnes and others [13].

Epidemiological data in the world, in particular in Ukraine, prove the increase in Lyme borreliosis incidence. The situation concerning TB also remains difficult, especially due to the spread of drug resistant TB. Ternopil region is endemic with Lyme borreliosis, due to the location in the zone of broad leaf and mixed forests. [3, 5, 15].

However, Ukraine has no data determining Borrelia burgdorferi infection in patients with granulomatous respiratory diseases, including sarcoidosis and tuberculosis. Thus, it is an urgent matter to examine the prevalence of Borrelia burgdorferi infection in patients with sarcoidosis and tuberculosis, because Lyme disease has many clinical features similar to sarcoidosis and tuberculosis (pleural lesions, heart, joints, nervous system, skin) [7, 8, 14].

Conclusions

The absence of appeals for medical care, lack of sufficient information on Lyme borreliosis and disuse of preventive measures for tick bite by the interviewed patients of Ternopil regional TB dispensary departments, proves the need of improvement of health education on Lyme borreliosis among this category of population. 24 (82.7%) of 29 respondents did not remember the tick bite.

Lyme borreliosis symptoms are similar to those of sarcoidosis and tuberculosis (pleural lesions, heart, joints, nervous system, skin), and tick bites give the reasons to examine these patients of Borrelia burgdorferi senso lato.

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RESEARCH IN SENSITIVITY TO ANTIBIOTICS, ANTISEPTICS IN *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM PATIENTS WITH INFECTIOUS COMPLICATIONS

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Background. *Infections caused by Pseudomonas are one of the topical issues of medicine.*
Objective. *The aim of the research was to study sensityvity to antibiotics, antiseptics of P. aeruginosa clinical strains that cause infectious complications in patients with burns.*
Methods. *Microbiological study of biological material, received from 435 patients with burns of the 3rd stages (2011-2015 years). In early terms of burn disease 127 clinical strains of P. aeruginosa were isolated from patients. Standard methods were used to identify clinical isolates of P. aeruginosa by their morphological, tinctirial, culture and biochemical properties. The research of antimicrobial action of antiseptics, antibiotics against Pseudomonas were carried out by means of standard methods according to the Directive of the Ministry of Health of Ukraine (No. 167 from 05.04.2007 p.) and guidelines of National Committee of Clinical and Laboratory Study (NCCLS, 2002).*
Results. *It was established that P. aeruginosa caused infectious complications in 23.9% of patients among other pathogens. Clinical isolates of P. aeruginosa were found to be low sensitive to amoxicillin/clavulanate (30.76%), ceftazidime (25.92%), cefoperazonum/sulbactam (46.15%), aztreonam (51.85%), tobramycin (38.46%), amicacin (70.34%), doxiciclini (26.92%), fluoroquinolones (59.26%). The analitical progistic criteria of decrease of sensitivity to ceftazidime, cefepim, meropenem and gatifloxacin were found in P. aeruginosa. This pathogen was determined to be sensitive to decasan®, antimicrobial composition of decamethoxine®, iodine pvidone.*
Conclusions. *Clinical strains of Pseudomonas aeruginosa, being highly resistant to antibiotics, are also very sensitive to antiseptics decasan®, antimicrobial of decamethoxine®, povidone iodine.*

KEY WORDS: **antibiotics; antiseptics; infectious complications; resistance.**

PUBLIC HEALTH AND EPIDEMIOLOGY

Introduction

Infection is a determinative factor of recovery of patients with different diseases in critical state. It is the main reason of severe complications and mortality among seriously ill patients. Infectious complications, caused by such non-fermenting Gram-negative bacilli as *Pseudomonas*, are one of the topical matter in contemporary medicine. The incidence of infectious complications, caused by *P. aeruginosa* has no tendency to decrease, despite the use of up-to-date methods of diagnostics and treatment of diseases with antimicrobial agents. Management of *P. aeruginosa* related infection is of great significance among burn patients [1, 2].

It is established that unsatisfactory results of prevention, treatment of infectious complications of burns are due to distribution of *P. aeruginosa* polyantibioticresistant strains in surgical clinics. environmental factors Active antimicrobial prevention and management by environmental factors provide selective effect on bacteria, which obtaines new qualities under this action (resistance to environmental factors, high virulence and resistance to antibiotics). Such qualities are determined by genome of microorganisms and can be caused by mutations that prove high adaptation to bacteria. In these cases, infectious complications caused by *P. aeruginosa* are still a current issue [3, 4].

Current principles of prevention and treatment of infectious complications in patients with burns include antimicrobials straight after clinical sympthoms are evidenced. So, wide spectrum antimicrobials are empirically used. Nevertheless in conditions of high resistance of microorganisms to antibiotics the absolute therapeutic effect cannot be achieved. Complex approach is one of the most perspective tendency in fight against *Pseudomonas* infection,

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it include combined use of antibiotics and antiseptics. That is why, the research in the effectiveness of antimicrobials against strains of *P. aeruginosa* that cause infectious complications is essential [5].

The aim of the research was to study sensitivity of *P. aeruginosa* clinical strains, isolated from patients with infectious complications, to antibiotics and antiseptics

Methods

Microbiological research of wound exudate samples taken from burn surfaces of 435 patients with deep burns of the 3rd stages took place in 2011–2015. All patients enrolled into the research had infectious complications. They were treated in burns centre of N. I. Pirogov Vinnitsya Regional Clinical Hospital. Microbiological research was carried out in bacteriological laboratory of the Department of Microbiology of Vinnytsya National Pirogov Memorial Medical University of the Ministry of Health of Ukraine. The research consisted of isolation of pure culture of pathogens identified by their morphological, tinctirial, culture and biochemical properties. The research samples were separated by sterile plug before antibacterial therapy. 645 strains of opportunistic bacteria were isolated from patients with burn trauma.

During the first 7 days of patients' treatment in the burn centre *P. aeruginosa* caused infectious complications in 23.9% of cases in comparison with other etiological pathogens. So the sensitivity of *P. aeruginosa* isolates to antibiotics and antiseptics was studied. Biochemical activity of clinical strains of *P. aeruginosa* was defined by means of test-system (NEFERMtest-24 (PLIVA – Lachema a. s., Chech Republic), which consisted of biochemical tests (indol, arginin, urease, lysine, glucose, fructose, inositol, saccharose, phosphatase, beta-galactosidase, beta-glucuronidase, N-acetyl-beta-D-glucosaminidase, mannitol, xylose, cellobiose, galactose, nitrates, nitrites, eskulin, gamma-glutamyltransferase, lactose, maltose, tregalose, citrate of Simonce). Cytocromeoxidase activity was additionally studied (OXY-test).

The study of bacteria sensitivity to antibiotics and antiseptics (decasan® (DS), miramistine (MR), chlorhexidine digluconate (CHD), povidon iodine (PI), antimicrobial composition of decamethoxine® (AMC) was conducted by standard methods according to The Directive of the Ministry of Health of Ukraine (No. 167 from 05.04.2007 p.) and guidelines of National Committee of Clinical and Laboratory Study (NCCLS, 2002). Sensitivity rate of *P.aeruginosa* clinical strains to antiseptics was evaluated by the value of minimal bactericidal concentration (MBcC, in mkg/ml) that was obtained by the serial broth dilution method [7].

In the research mathematical and statistical analysis of sensitivity of *P. aeruginosa* clinical strains to antibiotics, antiseptics were conducted. The methods of statistical analysis, used in the research, enabled definition regular relationship between numeric values of variable indications and probability of realization of these values in array observations. To find out the effectiveness of antibiotics current methods of mathematical prediction were used. So, accurate sensitivity of *P. aeruginosa* and its extrapolation on the studied system by building a series of standard normative mathematical models of predicted sensitivity of the pathogen to antibiotics was foreseen. Thus, analytical methods with concretization of absolute and relative optimum meanings were used.

Predictive models of *P. aeruginosa* sensitivity to antibiotics of different groups were investigated as summation of mathematical formulas, which were used to determine these qualities of microorganisms accordingly to their meanings, extrinsic and initial conditions and time. The significance of the results, possible sensitivity rate to antimicrobials was assessed by the coefficient of determination (r^2). The obtained data underwent computer processing by means of original programs "STATISTICA 7"; "Matlab 7.11". For each group of microorganisms strains for each year of study arithmetic mean (M), error arithmetic mean (m), standard deviation (σ) were defined. Using the methods of applied mathematics the approximation and interpolation of the data were conducted. Due to this analytic dependence the possible dynamic of sensitivity rate of *P. aeruginosa* strains, isolated from the patients, was estimated [8].

Results

As the result of the research, 154 clinical strains of *P. aeruginosa* were isolated and identified that looked like typical bacteria of this specimen due to their tinctirial, morphological, and cultural properties. Strains of *P. aeruginosa*, isolated from the patients, had typical biochemical features (Table 1).

All clinical isolates of *P. aeruginosa* (100%) produced haemolysins, oxidase and decomposed D-mannitol.

As the results of the research, it was proved that *P. aeruginosa* was sensitive to aminopenicillin

Table 1. Enzymatic properties of isolated strains of *P. aeruginosa* (n 154)

Test	*P. aeruginosa*
Oxidase	+
Catalyse	+
D- cellobiose	-
Glucose	+
y- glutamyltransferase	+
D-mannitol	+
L_1-prolinarylamidase	+
Lipase	-
Urease	+/-
Malonate	+
Phosphatase	+/-
L- lactate	-
N-Acetyl-beta-D-Glucosaminidase	-
D- mannose	-
L- histidine	-

combined with amoxicillin/clavulanate only in 12.30–30.76% of cases (Fig. 1).

The study of cephalosporins effectiveness showed that sensitivity of *P. aeruginosa* clinical isolated to ceftazidime had not exceeded 25.92% for five years of our study. The efficacy of ceftazidime was decreased by 8.5% in 2014–2015. By means of mathematical analysis the

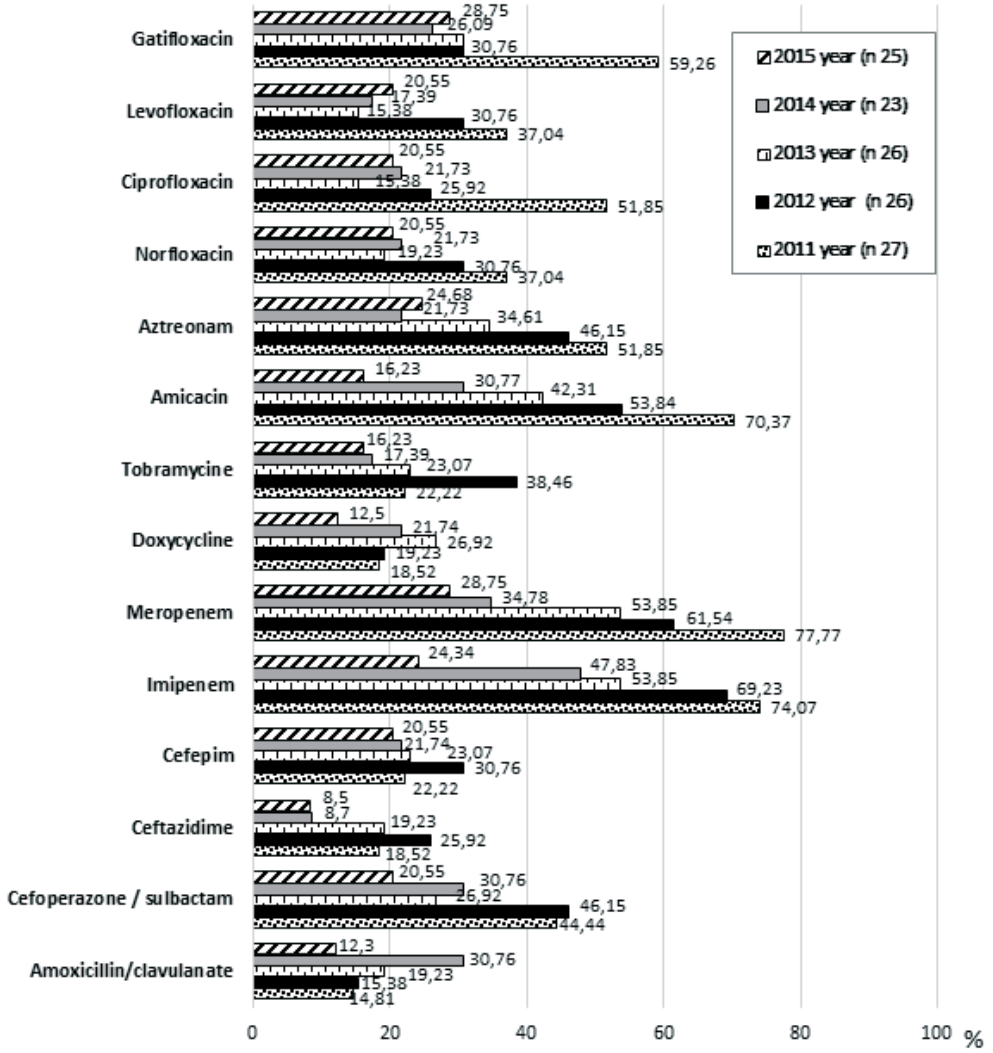

Fig. 1. Sensitivity of *P. aeruginosa* isolates, received from seriously ill patients with burns (%).

predicted decrease of *P. aeruginosa* sensitivity to ceftazidime has been proven (Formula 1; Fig. 2).

$$\text{Ceftazidim} = \frac{1}{a + bx\ln x + c\ln x / x^2}, \quad (1)$$

a=-55358.47; b=2.2527981; c=1.1111315e+10

According to the results of the study of cephalosporins activity, the efficacy of cefepime was not proved (20.55–30.76%). Prognostic curve of sensitivity of *P. aeruginosa* clinical strains to cefepime could refer to parabolic function, presenting future decrease of the efficacy of this antibiotic. It was found out that *P. aeruginosa* colonized at burn surfaces was resistant to cefalosprins combined with sulbactam. Only 20.55–44.44% of *P. aeruginosa* clinical strains were sensitive to cefoperazone/sulbactam.

In 2011–2015, the tendency of decrease of *P. aeruginosa* clinical strains sensitivity to meropenem (28.75%), imipenem (24.34%) was registered. Predictive analysis of *P. aeruginosa* sensitivity to meropenem was revealed by its linear decreasing in 2011-2015 (Formula 2; Fig. 3):

$$Meropenem = a + bx3 + cx/\ln x, \quad (2)$$

a=1719659.7; b=8.4437694e-05; c=-9101.5317

Similar decrease of *Pseudomonas* sensitivity to imipenem was defined in the research.

The estimation of *P. aeruginosa* sensitivity to aminoglycosides proved low efficacy of tobramicin (16.23–38.46%). The decrease of *P. aeruginosa* sensitivity to amikacin was also evidenced in the research (Formula 3; Fig. 4).

$$Amikacin \quad y = a + bx + cx^{2,5} + \frac{d}{x^{1,5}}, \quad (3)$$

a=-5.8244079e+9; b=2892515.8; c=-8.0030078; d=1.3157016e+14; x – year

Thus, *P. aeruginosa* clinical strains were found to be significantly sensitive to amikacin in 2011 (70.37%). But in 2015 the number of *P. aeruginosa* sensitive strains was significantly lower (16.23%). Analytical prediction revealed linear decrease of *P. aeruginosa* sensitivity to amikacin.

It was found out, that isolates of *P. aeruginosa* were of low sensitivity to aztreonam (34.61–51.85%). Five-year-study of *P. aeruginosa* sensitivity to aztreonam presented its depression (21.73%) (Formula 4; Fig. 5).

$$Aztreonam \quad y = a + bx2 + cx4 + dx6 \quad (4)$$

a=-6.5573499e+8; b=485.57507; c=-0.000119856; d=9.861468e-12; x – years

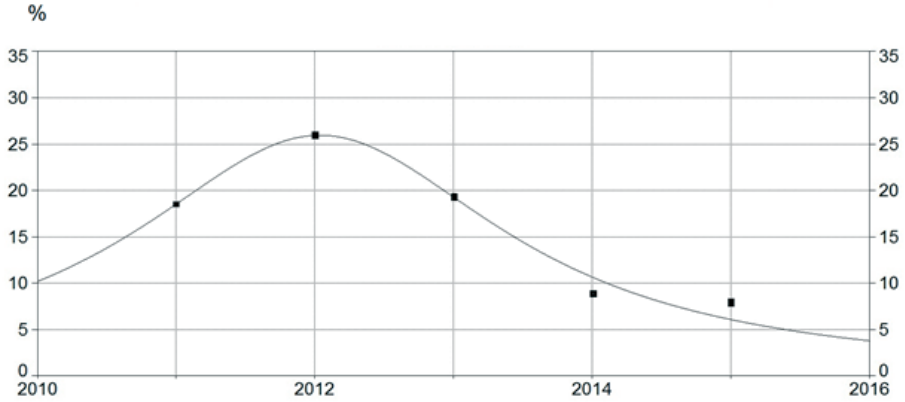

Fig. 2. Predictive sensitivity to ceftazidime of *P. aeruginosa* clinical strains, received from the patients with burn wounds (%).

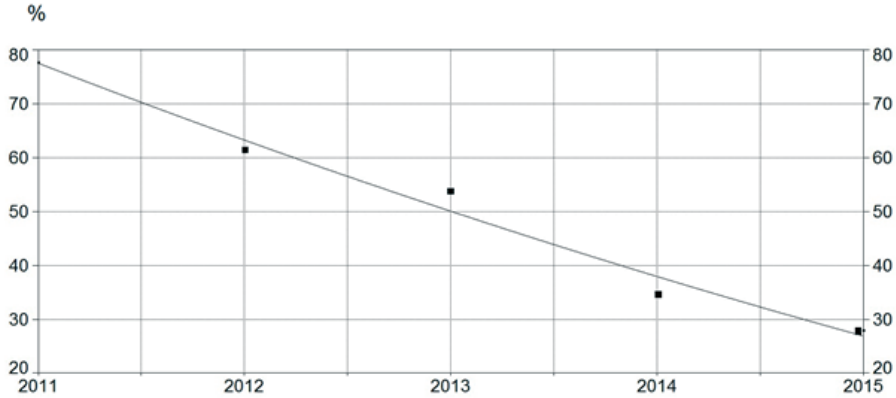

Fig. 3. Predictive sensitivity of *P. aeruginosa* strains to meropenem (%).

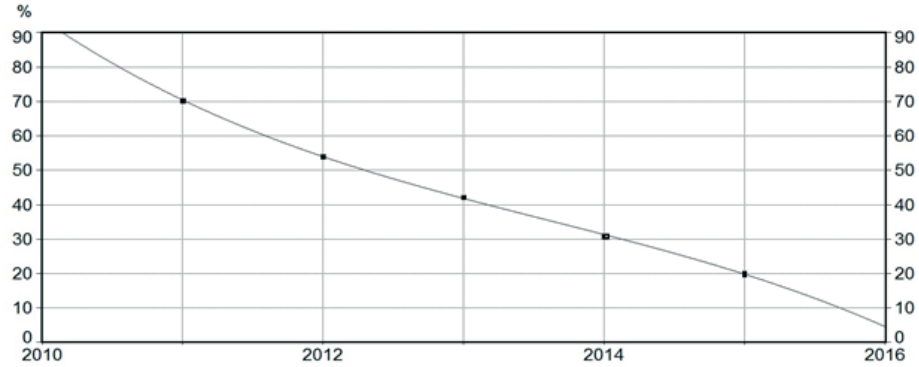

Fig. 4. Predictive sensitivity of *P. aeruginosa* strains to amikacin (%).

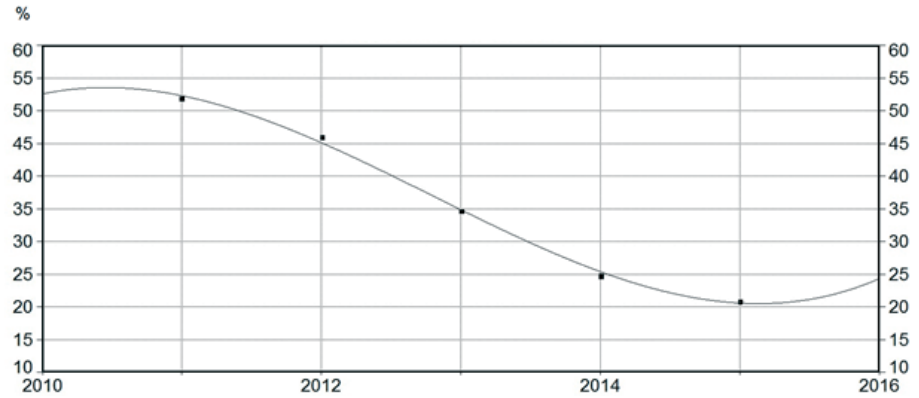

Fig. 5. Predictive sensitivity of *P. aeruginosa* strains to aztreonam.

In *P. aeruginosa* clinical strains of low antibiotic sensitivity, different sensitivity rates to antiseptic remedies were found (Table 2).

In the research, clinical strains of *P. aeruginosa* were found to be sensitive to AMC (MBcC 53.15±1.27 mkg/ml). Inhibitory effect of DC® (46.65±0.76 mkg/ml), MR (47.83±2.04 mkg/ml) did not significantly differ from AMC ($p>0.05$). Clinical strains of *P. aeruginosa* were resistant to ChD. Thus, MBcC of ChD versus *P. aeruginosa* exceeded 165.85±10.9 mkg/ml that proved its low efficacy against these bacteria. DC® and MR had in 1.8 times higher antimicrobial qualities versus *P. aeruginosa* in comparison with ChD ($p<0.001$). Antimicrobial activity of AMC was 3 times higher than in ChD ($p<0.001$). Antibiotic resistant clinical strains of *P. aeruginosa* were studied to obtain high sensitivity to bactericidal concentrations of povidone iodine that is contained in the official pharmaceutical form of this remedy.

Discussion

However, in the research the obtained data proved that the amount of isolates of *P. aeruginosa* in general population was high and reached 23.9% of all microorganisms, isolated from burn wounds in patients, who suffered from infectious complications. Such tendency did not differ from established trend of the increasing importance of *P.aeruginosa* as a prominent pathogen of infectious complications [2, 5].

The results of the research proved low sensitivity of *P. aeruginosa* clinical strains to antibi-

Table 2. Antimicrobial activity of antiseptics against *P. aeruginosa* isolates

Antiseptic	Minimal inhibitory concentrations	Minimal bactericidal concentrations
	(M±m), mkg/ml	
Antimicrobial composition of decamethoxine® (AMC)	31.0±0.17	53.15±1.27
Decasan® (DC)	46.65±0.76†	91.34±1.69*
Miramistin (MR)	47.83±2.04†	96.85±3.25*
Clorhexidine digluconate (ChD)	106.3±4.76***	165.85±10.9***
Povidon iodine	3858.27±192.13***	6742.13±397.27***

Notes. † – $p>0.05$, * – $p<0.05$ ** – $p<0.01$, *** – $p<0.001$ in comparison with AMC.

otics of different groups. The low sensitivity of *Pseudomonas* to antibiotics, containing beta-lactam ring in their molecule, was defined. However, in some clinics these antibiotics are widely used as agents of choice for prevention and treatment of infectious complications in patients with burns. Low efficacy of cephalosporins against *P. aeruginosa* according to their sensitivity rate was also determined. Higher sensitivity rate to carbapenems was proved in *P. aeruginosa* (74.07–77.77%). The estimation of the antibiotic sensitivity of *P. aeruginosa* to aminoglycosides showed their low effectiveness (16.23-38.46%). It was proved, that *P. aeruginosa* isolates, received from the patients with deep burns, were of low sensitivity to aztreonam (34.61–51.85%) [3, 4].

Bactericidal qualities of antiseptics were established that proved high efficacy of decamethoxine, decasan, povidone iodine against clinical strains of *P. aeruginosa*, as pharmaceutical forms contained in adequate doses of these drugs. In the majority of cases bactericidal concentrations of miramistin exceeded the dose in the remedy officinal form [2, 6].

Conclusions

Clinical strains of *P. aeruginosa*, which cause infectious complications in seriously ill patients with burn disease, have moderate sensitivity to beta-lactam antibiotics (amoxicillin/clavulanate – 30.76%; ceftazidime – 25.92%; cefoperazone/sulbactam – 46.15%; meropenem – 77.77%; imipenem – 74.07%; aztreonam – 51.85%); aminoglycosides (tobramicin – 38.46%; amikacin – 70.34%).

Analytical models predict parabolic decrease of sensitivity of *P. aeruginosa* to ceftazidime, cefepime; lineal tendency of decrease of their sensitivity to meropenem, imipenem.

Clinical strains of *Pseudomonas aeruginosa*, being highly resistant to antibiotics, save their high sensitivity to antiseptics decasan®, antimicrobial composition of decamethoxine®, povidone iodine. The use of antiseptics is very promising in prevention and treatment of infectious complications caused by *P. aeruginosa*.

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MORPHOLOGICAL STUDY OF COMMUNICATION BETWEEN THE LONGITUDINAL STRIPS AND THE HUMAN CORPUS CALLOSUM

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Background. *It is established that there is an anatomical relationship between the corpus callosum and longitudinal strips. These formations must correlate to the common commissural system of the corpus callosum conductors. At present this issue in such a formulation is not considered in literature on Neuromorphology.*

Objective. *The study was aimed to determine the commutations between the longitudinal strips and commissural conductors of the corpus callosum.*

Methods. *The corpus callosum of people aged from 36 to 60 was studied. Some slices of the corpus callosum stem were used for impregnation and then inserted in paraffin blocks. Another part of these slices was subjected to plastination in epoxy resin.*

Results. L*ateral longitudinal strips contain significantly greater mass of nerve conductors. Most of them compactly pass along limbic ring, while the other part is combined to nerve fibres of the corpus callosum commissural cords. The longitudinal strips are coated with an outer limiting glial membrane (grey coating).*

Conclusions. *By means of the corpus callosum the connections between conscious and subconscious brain areas are structurally fixed. It can be assumed that longitudinal strips have relation to hippocampal area, related to the ancient formation of the pallium. This does not exclude the fact that the nerve fibres, found within longitudinal strips may have projections on the cortical cells of vaulted gyrus, which is considered to be paleopallium sphere. So, this interaction between the ancient and the old cortex should presumably be carried out by means of lateral longitudinal strips.*

KEY WORDS: **the corpus callosum; lateral longitudinal stripes; medial longitudinal stripes.**

Introduction

It is established that there is a direct anatomical connection between the corpus callosum and such formations as longitudinal strips (medial and lateral), fornix and transparent partition [1, 4-7]. According to current researches, they are considered to be conductive formations of limbic brain. It is established that the longitudinal strips are thin strands of nerve fibres encircling upper surface of the corpus callosum, which anteriorly are connected to subcallosal gyrus, and posteriorly under the splenium reach the hippocampal area of dentate gyrus. This fact significantly defines the closed circular structure of limbic brain.

Due to this fact, it is clear that these formations in some way should be linked to a common system of commissural conductors of the corpus callosum itself. Despite this evidence, in the current literature on Neuromorphology this issue, in this formulation, has not been even considered. This fact can be surprising, because of its importance in understanding the mediating links in the associative interaction between conscious and subconscious psychic spheres.

We actually started studying these structures in this aspect in our previous research [2] and brought to notice the shape of upper surface of the corpus callosum, having two noteworthy sublitities, known in the literature as the transverse and longitudinal strips. Firstly, we have proved that so called transverse strips are actually an external protrusion of constituent formations of the corpus callosum that we termed as commissural cords. The wavily curved longitudinal strips run on their spindle-shaped elevations, and a pair of them in contralateral position occupies borderline position

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between the free part of the corpus callosum and the medial surface of cingulate gyri. In the intermediate position between them there is a pair of closely spaced similar formations, which are called medial longitudinal strips. Besides the detailed description of their form that is provided above in this article, we mention only one significant feature that evidences that these longitudinal strips do not just lie on the upper surface of the corpus callosum but are closely related to it. This is indicated by periodically occurring local immersions of longitudinal strips in the thickness of the corpus callosum in recesses between transverse spindle-shaped elevations that gives them the shape of suture stitches. Taking into consideration this persuasive anatomical fact, it is not enough to be absolutely sure in the presence of commutation relations between these formations and commissural conductors of the corpus callosum. To answer this question some additional histological examinations were required.

Our study was aimed to determine the commutation relations between longitudinal strips and commissural conductors of the corpus callosum.

Methods

In this research we used whole mounts of the corpus callosum of 5 men and 5 women aged from 36 to 60 years, which were separated from whole brain mounts after their two-week fixation in 10% neutral formalin. These mounts were received due to the agreement between Kharkiv National Medical University and Kharkiv Regional Bureau of Forensic Medical Examination.

The mounts were used to excise plate slices of standard thickness (2 mm) out of corpus callosum stem. In addition, their excision was performed in two mutually perpendicular directions: lengthwise and crosswise. Some of them were used for impregnation and further embedding in paraffin blocks, according to the conventional methods. Later they were used to prepare serial slices with traditional hematoxylin and eosin, and Van Gieson staining.

The other part was subjected to plastination in epoxy resin using the epoxy adhesive Khimkontakt-Epoksi, according to the method developed at the Department of Human Anatomy of UMSA (Poltava) [3].

At the end of the last stage of impregnation in pure epoxy resin these plate mounts were placed between two glass plates, isolated with plastic spacers (to prevent adhesion of mounts and glass). In such a 'sandwich' mounts were thrust together with sparing clips and in this way they were subjected to final polymerization and acquired flattened, but not deformed shape.

After complete polymerization these palatinate mounts were used for making thin sections that were stained with a 1% solution of methylene blue per 1% solution of borax.

The study of all mounts (paraffin slices and thin epoxy sections) was carried out using a binocular microscope MBS-9 and Konus microscope with a digital photo-adaptor.

Results

When staining thin epoxy sections of the corpus callosum with methylene blue it was found out that lateral longitudinal strips, which shape is of pointed hills in cross-section, have basophilia of the same intensity as that of its main stratum. However, there is a layer of a light coloured matter between them, giving the reason to assume certain detachment between them (Fig. 1) and we have to find out the extent of this detachment.

Medial longitudinal strips located in the medial position of corpus callosum cross section look somewhat different (Fig. 2). Because of their irregular location on the surface of spindle-shaped elevations, that was noted in the introductory part of this article, in cross-sectional perspective they are not always detected as paired or equal in terms of their altitude formations. Therefore, the density of their connection with the substance of the corpus callosum is a kind of an ambiguous judgment. For example, in some of its epoxy slices (in cross section) they look like elevations of asymmetrical height and their substance (in methylene

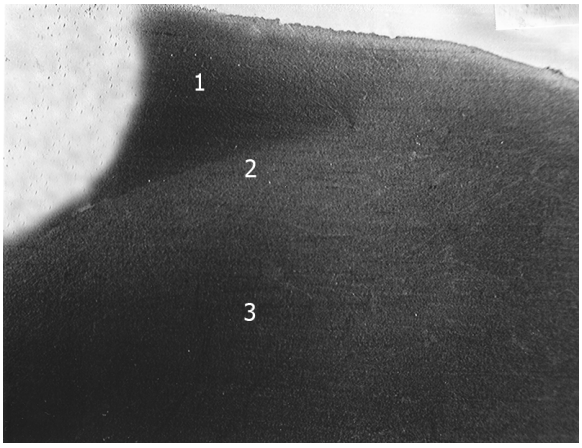

Fig. 1. Lateral cross-section of an adult male corpus callosum. Epoxy slice. Painted in methylene blue. x7.
1 – lateral longitudinal strip in cross-section; 2 – intermediate area; 3 – the substance of the corpus callosum.

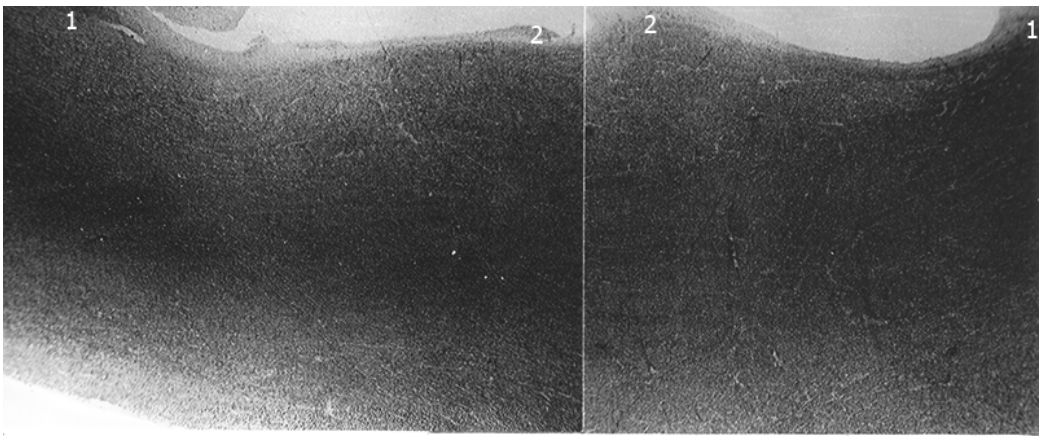

Fig. 2. The cross-section of the corpus callosum of a 56-year-old male. Epoxy slice. Painted in methylene blue. x4.
1 – lateral longitudinal strips; 2 – medial longitudinal strips.

blue staining) has less pronounced basophilia compared to the basic thickness of the corpus callosum (Fig. 2). In addition, there is a poorly pronounced intermittent light streak between the corpus callosum and medial strips.

These tinctorial differences between the substance of medial longitudinal strips and the actual corpus callosum can be distinctly detected on paraffin slices when stained by Van Gieson (Fig. 3). First of all it should be noted that both substances are inseparably adjusted to each other, but between them there is a visible intermediate area in the form of a narrow band of condensed cellular elements. Inside them there are interfascicular oligodendrocytes appearing not only in the actual corpus callosum, but also in the substance of medial strips, with the only difference: in the latter they are present in more rarefied state. But the most significant feature is that these glial cells are arranged in rows oriented into the thickness of the corpus callosum, thereby creating the idea that both these cells and nerve fibres accompanied by them pass from medial strips to the latter.

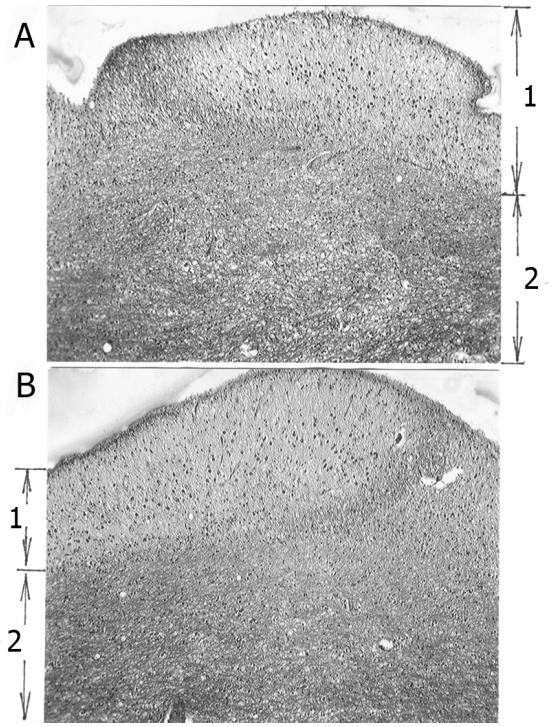

Fig. 3. The medial longitudinal strips of corpus callosum of an adult man (A) and a woman (B) in cross section. Paraffin slices. Van Gieson staining. x10.
1 – medial strips; 2 – the corpus callosum.

Discussion

This fixed morphological pattern is not enough for unambiguous judgement about the real commutation direction of nerve conductors. So we have to limit ourselves to the general conclusion that medial strips are in close connection with the corpus callosum, either by means of nerve fibres exchange between them, or as a result of their transition from medial longitudinal strips to the corpus callosum. But in fact, in either case it is impossible to establish the proportion of the conductors is involved.

Now let's discuss the lateral longitudinal strips, which are closely adjusted to subjacent matter of the corpus callosum as well as the medial ones, but between them, unlike the former, there is a more pronounced layer of a less densely coloured substance. Paraffin slices, when stained with haematoxylin and eosin by Van Gieson, allow us to define that this enlightened layer entirely relates to the limits of lateral longitudinal strips, because between them and the substance of the corpus callosum there is a dividing strip of compacted substance due to the increased concentration of myelinated nerve fibres in this place (Fig. 4). On the lower microphotograph of this figure one can

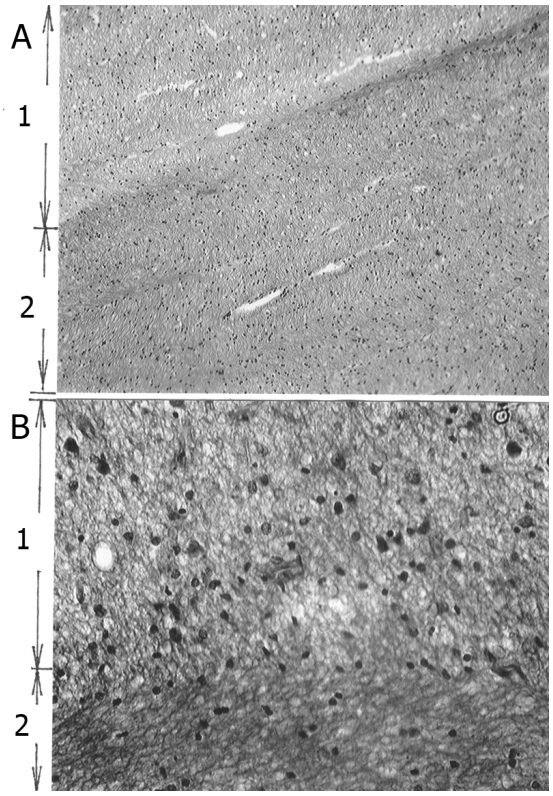

Fig. 4. The border zones between lateral longitudinal strips and the corpus callosum in cross-section. Paraffin slices. A – Van Gieson staining, x10; B –hematoxylin and eosin staining, x40.
1 – lateral strip substance; 2 – the substance of the corpus callosum.

see that this border concentration of nerve fibres seems to appear due to the replenishment of fascicular portions of corpus callosum with nerve conductors penetrating inside them from the enlightened area of lateral strips. In turn, the latter are the nerve fibres, which are separated along their way from the dense mass of longitudinal lateral strips. Although this fact seems obvious, as in the case of medial longitudinal strips, we still have no reason to exclude the fact of their mutual exchange of nerve conductors. Still, on the basis of the data received it is possible to make a presumptive conclusion that lateral longitudinal strips, unlike other similar to them the medial ones, contain a much greater mass of nerve conductors. Most of them in compact form pass in transit manner along limbic ring, while the other part of them, which is less numerous, combines with the mass of nerve fibber's of the corpus callosum commissural cords.

Additionally to this data, it should be noted that both the medial and lateral longitudinal strips are coated with organically connected outer limiting glial membrane, known in the literature as grey coating which has a darker tone compared to white brain matter due to minimum amount of lipoid substances therein in the form of myelin.

Conclusions

We did not aim at understanding the details of the links between the associative conductors of limbic brain, distributed on lateral and medial longitudinal strips, and the commissural commutations of the corpus callosum, because a research like this requires a lot of efforts, not only by neuromorphologists but also neuroscientists and psychiatrists. The aim of our study was to show that the existing concept of the corpus callosum as a purely adhesive formation between the contralateral cortical areas of the new pallium is far from being complete. The results of our study give evidence that by means of the corpus callosum the connections between conscious and subconscious brain areas are structurally fixed. It can be assumed that the longitudinal strips spanning the corpus callosum on its upper surface are mediatingly related to hippocampal area, related to the formation of ancient pallium. This does not exclude that nerve fibres found in longitudinal strips may have projections on cortical cells of vaulted gyrus, which is considered to be the paleopallium sphere. If that is the case, then the interaction between ancient and old cortex should presumably be carried out by means of lateral longitudinal strips.

But, as mentioned in the introduction, this is not limited to the anatomical structures of limbic brain, having a close relationship with the corpus callosum. Apart from the former (longitudinal strips) and opposite to them, that is, at the bottom side, two other formations are coalesced with the corpus callosum. They are a transparent partition and fornix, which, except for their anatomical descriptions and considering them as conductive constituent of limbic brain, are represented in the literature neither for the presence nor absence of a direct connection of their conductors with commissural fibres of the corpus callosum. Resolving of this issue will be presented in the next report.

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MORPHOLOGICAL RESEARCH ON FREE-RESIDUE OXIDATION PROCESSES IN CASES OF DECIDUAL CELLS OF PLACENTA IN CHORIOAMNIONOTIS AND BASAL DECIDUITIS COMBINED WITH IRON-DEFICIENCY ANEMIA IN THE PREGNANT

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Background. *The oxidative modification of proteins is lately pivotal to pathologists and it is a new way of research on different pathological conditions, as well as the diagnostics of inflammation processes in placenta.*

Objective. *The study was aimed at the research of nitro peroxides and establishing the specific features of oxidative modification of proteins in inflammation of placenta with iron deficient anaemia in the pregnant.*

Methods. *Chemiluminescent and histochemical technique (with bromphenol blue on 'acidic' and 'basic' proteins according to Mikel Calvo) was applied.*

Results. *The intensity of nitro peroxides glow in chorioamnionitis and basal deciduitis increased in comparison with the samples of physiological and iron deficient anaemia gestation. At the same time in chorioamnionitis the glow intensity is higher than in basal deciduitis.*

Due to the results of immune histochemical technique held while analysing the samples, together with chorioamnionitis and basal deciduitis the R/B increases and in basal deciduitis the rate, is probably, higher, than in chorioamnionitis. At the same time, the extent of oxidative modification of proteins in cases of inflammation with iron deficient anaemia in the pregnant is on the average higher than with no iron deficient anaemia in the pregnant.

Conclusions. *High level of nitro peroxides in placentae basal plate in secundines inflammation, the increase in R/B rate, in other words the prevalence of 'acidic' proteins over 'basic' ones, is evidenced due to the increase of the intensity of oxidative modification processes of proteins in cases of deciduitis.*

KEY WORDS: **inflammation; placenta; anaemia; iron-deficiency.**

Introduction

The formation of active forms of oxygen and oxidative modification of macromolecules is usual and important biological processes. But the creation of oxygenous residues excess could damage cells and facilitate the development of many illnesses [1], and in certain cases, a molecular pathology of the organism [2].

The oxidative modification of proteins is lately pivotal to pathologists [3], and it is a new way of research on different pathological conditions [4], as well as the diagnostics of inflammation processes in placenta [5]. In this area the scholars used a range of techniques aimed at detection of nitro peroxides [6], as the most responsive and long lasting oxidants among free residues, and at estimation of the degree of protein oxidative modification in various structures of placenta [7]. However, this aspect in combination with iron deficient anaemia in the pregnant has not been explored yet [8] and has been being studied that is the objective of our study.

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Objective

Chemiluminescent technique with luminol was applied in the research of nitro peroxides in cases of inflammation. The aim of the study was to establish the specific features of oxidative modification of proteins in cases of deceduitis of basal plate in long lasting basal deciduitis and chorioamnionitis in combination with iron deficient anaemia and without it in the pregnant by means of immune histochemical and micro spectral photometric procedure.

Methods

The control group consisted of placenta (n=20) received at the urgent delivery of clinically healthy women in childbirth and in cases of iron-deficiency anaemia in gravidas (n=21). Experimental group consisted of the placenta (n=123) received from women in childbirth at gestation period of 37-40 weeks with chorioamnionitis and basal deciduitis combined with iron deficiency anemia in gravidas and without it.

The first procedure (a chemiluminescent technique) was performed on the frozen sections of placenta. The luminol originated chemiluminescence was studied under the luminescent microscope LUMAM P8. On digital photomicrographies, obtained by means of computer estimation of glow intensity in a 256 gradations scale: from 0 (glow absence indicator) to 255 (glow top intensity), the quantitative measuring of luminescence was carried out [8].

For validity estimation of averages contrast Student's t-test was used, after positive verification of the samples for normal distribution in it was determined by the Shapiro-Wilk test (computer program PAST 3. 14, free license, O. Hammer, 2016) [9]. For approval or rejection of statistic hypothesis the significant difference $p \leq 0.05$ was used.

To evaluate the extent of oxidative modification of proteins, Histochemical technique was carried out with bromphenol blue on 'acidic' and 'basic' proteins according to Mikel Calvo [10].

Optic images were converted into digital using the digital camera and were analysed with licensed copy of the ImageJ computer program (1.48, W. Rasband, National Institute of Health, USA) using probe computer spectrometry in the system of colour analysis RGB. According to the designated system of colour analysis the intensity of red and blue colours in coloration was assessed. According to the Mikel Calvo technique, red coloration matches carbonyl groups, whereas blue coloration matches protein amines; therefore establishment of mathematical relation between coloration intensity in two colours (spectrum areas) the R/B was received and the extent of oxidative modification of proteins was evaluated (relation of carboxylic and amines) [11,12].

Results

Chemiluminescent glow of nitro peroxides was evaluated and the level of physiological gestation of placentae was estimated in cases of iron deficient anaemia in the pregnant. The averages are introduced in Table 1.

The results of quantitative indicators of chemiluminescent glow of nitro peroxides and the R/B rate in cases of deciduitis of placenta basal plate in acute and long lasting chorioamnionitis, and basal deciduitis in combination with iron deficient anemia in the pregnant are presented in Table 2.

Discussion

According to the data above, we should emphasise that in physiological gestation of pregnancy in cases of iron deficient anaemia, significant deviations in any conducted technique was not evidenced. The data in Table 2 proves that in chemiluminescent research technique the intensity of nitro peroxides glow in cases of chorioamnionitis and basal deciduitis increases in comparison with the samples of physiological and iron deficient anaemia gestation. The fact that, in cases of placentitis with underlying iron deficient anaemia in the pregnant, the quantitative indicators are higher than with no anaemia. At the same time, in cases of chorioamnionitis, the glow intensity is higher than in basal deciduitis.

Table 1. The quantitative indicators of chemiluminescent nitro peroxide glow and R/B rate (histochemical technique on 'acidic' and 'basic' proteins with bromphenol blue according to Mikel Calvo) in cases of deciduitis of placenta basal plate in physiological gestation and iron deficient anaemia in the pregnant

Basal pate structures	Research groups			
	Observation of physiological gestation (n=20)		Observation of iron deficient anemia in the pregnant without inflammation of secundines (n=21)	
Decidual cells of basal plate	Chemiluminescent glow of nitro peroxides with luminol	Histochemical technique on 'acidic' and 'basic' proteins with bromphenol blue on Mikel Calvo	Chemiluminescent glow of nitro peroxides with luminol	Histochemical technique on 'acidic' and 'basic' proteins with bromphenol blue on Mikel Calvo
	34±3.8	1.04±0.008	38±4.2 p>0.05	1.06±0.009 p>0.05

Table 2. Quantitative indicators of chemiluminescent glow of nitro peroxides and the R/B rate (histochemical technique on 'acidic' and 'basic' proteins with bromphenol blue on Mikel Calvo) in cases of deciduitis of placenta basal plate in acute and long lasting chorioamnionitis and basal deciduitis in combination with iron deficient anemia in the pregnant

Research groups	Chemiluminescent glow of nitro peroxides with luminol		Histochemical technique on 'acidic' and 'basic' proteins with bromphenol blue on Mikel Calvo R/B rate (M=m)	
	Secundines inflammation	Secundines inflammation in cases of iron deficient anaemia in the pregnant	Secundines inflammation	Secundines inflammation in cases of iron deficient anaemia in the pregnant
Acute chorio-amnionitis	154±4.9 (n=20)	186±5.1 (n=21) p=0.003	1.24±0.011 (n=20)	1.64±0.016 (n=21) p<0.001
Acute basal deciduitis	130±4.4 (n=21)	164±4.5 (n=20) p=0.002	1.89±0.015 (n=21)	2.14±0.018 (n=20) p<0.001

The photomicrographs of histological images give an idea of how some structures of placental basal plate look like, decidual cells in particular, when the immunohistochemically technique for 'acidic' and 'basic' proteins according to Mikel Calvo is applied (Fig.1).

When assessing visual histochemical preparations, decidua cells are clearly stained that is applicable for quantitative research, cell boundaries are defined by clear cell membrane colouring and contrasting colour around decidua cells fibrinoid. Nuclei and nucleoli were visualized fairly well. 'Basic' proteins prevailed in nucleoplasm, while 'sour' in the nucleolus.

The decidua cells cytoplasm specific colour was mostly granular in nature and spectral characteristics and optical density of colour varied greatly.

Relatively invariable data in assessing the degree of oxidative modification of proteins in placentas of physiological gravidity and gravidas are observed, which can be interpreted as the manifestation of involutory changes in placenta. The R/B factor is only slightly higher than what can be evaluated as a slight predominance of 'sour' proteins over 'basic' ones. These figures are important in terms of the R/B factor assessing, as an indicator of oxidative modification of proteins. However while analysing the samples with chorioamnionitis and basal deciduitis the R/B increases and in basal deciduitis the rate is probably higher than in chorioamnionitis. At the same time, the extent of oxidational modification of proteins in cases of inflammation in combination with iron deficient anaemia in the pregnant is on the average higher than with no iron deficient anaemia in these patients.

Taking into account our findings of high level of nitro peroxide in basal lamina of placen-

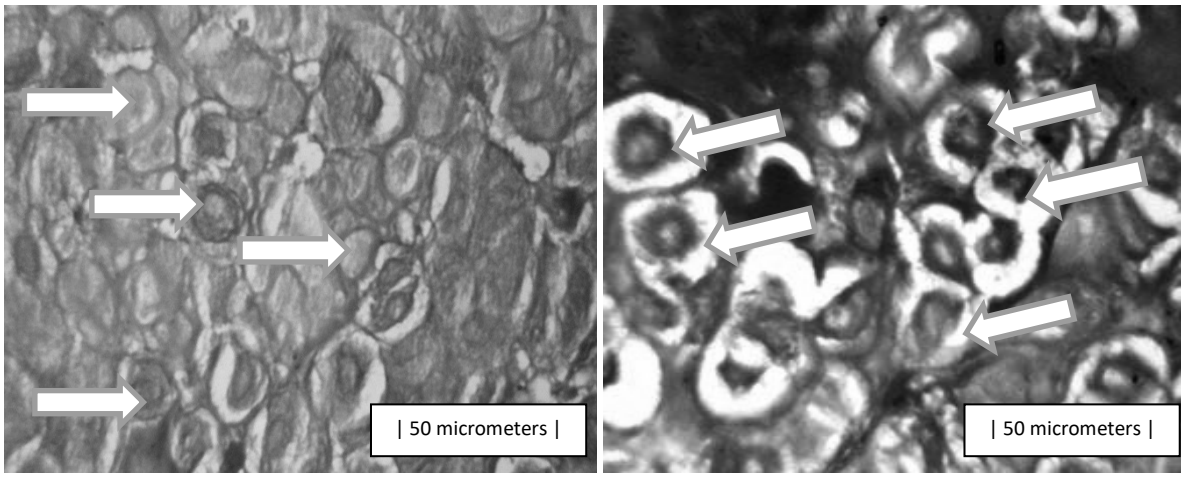

Fig. 1. Photomicrograph of placental basal plate. Histochemical technique for 'acidic' and 'basic' proteins with bromphenol blue according to Mikel Calvo. On the right – observation during physiological pregnancy. On the left – in cases of chronic deciduitis in placental basal plate.

tas which are characterized by secundines inflammation, increase in, i.e. the prevalence of 'sour' proteins over 'basic' ones; correlation between the increasing intensity of oxidative modification of proteins in cytoplasm of deciduitis and secundines inflammation would be reasonable.

Conclusions

Due to the chemiluminescent technique of nitro peroxides evaluation and immune histochemical technique of 'acidic' and 'basic' proteins assessment in cases of deciduitis of placenta basal plate, the impetuous increase of indicators is observed.

The inflammation of secundines in combination with iron deficient anaemia in the pregnant was evidenced by higher average rates than with no anaemia.

Considering the received data that proves high level of nitro peroxides in placentae basal plate in secundines inflammation, the increase in R/B rate, in other words prevalence of 'acidic' proteins over 'basic' ones, is evidenced due to the increase of intensity of oxidative modification processes of proteins in cases of deciduitis.

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MORPHOLOGICAL CHANGES IN LUNGS, HEART AND LIVER CAUSED BY EXPERIMENTAL ASSOCIATED CHEST AND THIGHS TRAUMA

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Background. *According to preliminary results obtained, the development of hypoxemia caused by chest trauma affected lipid peroxidation stimulation leading to disruption of cell membranes structure and consequently cell death closing the 'vicious' circle, which in our opinion and according to the literature cause multiple organ failure.*

Objective. *The aim of our study was to determine the features of morphological changes in cases of heart, lungs, liver injury after associated chest and both thighs trauma in rats.*

Methods. *For histological examination the pieces of left ventricle tissues of heart, lungs and liver were used, which were fixed in 10% neutral formalin solution, dehydrated and embedded in paraffin blocks.*

Results. *It was established that associated trauma of chest and both thighs in 1 day after the injury is accompanied by disturbances of hemodynamics in parenchymal organs due to venous congestion that occurs during the development of cardiovascular disease caused by traumatic shock. In the most severe period of maximum likelihood of complications, within 3 days, respiratory hypoxia, cardiovascular disorders, metabolic acidosis, and induced programmed cell death may develop.*

Conclusions. *On the background of increasing pulmonary oedema (thickening of inter air sac membranes) caused by associated trauma of chest and both thighs in rats after only one day of the experiment hemodynamic changes in heart (low-blood supply of vessels in epicardium and isolated blood vessels of venous type) and liver (mainly plethora of blood vessels) were observed, which lead to morphological changes of myocardium within 3 days.*

KEY WORDS: **trauma; lungs; liver; heart; morphological changes.**

Introduction

Trauma is the main cause of death among people of all ages, with most injuries that can be prevented by adequate quality diagnosis and correction. Over the past decade, due to increasing urbanization and mechanization of life, injuries have become an urgent social and economic problem. Multiple injuries, especially in road accidents, according to their frequency and consequences of social significance, are very important in the context of nosology forms leading to death and disability of victims and reduce labour resources of the country [1, 2]. The level of injury in the regions of Ukraine is growing steadily, but from injuries in 2001 40 thousand people died, and 2.3 million were injured. According to the State Statistics Committee of Ukraine, mortality, because of accidents, poisonings and injuries, takes the third place, and because of only injuries – the fourth among all causes [3, 4].

In recent years, a steady trend towards complications of traumatic injuries is evidenced, most of them are multiple and combined with the inherent nature of mutual burdening syndrome [5]. Combined traumatic injuries due to their complexity are especially dangerous and provide the significant threat to human life. [6] They are still unsolved problem for the world of medical science and health [7].

From the pathophysiological point of view, the risk of trauma is due to the involvement of target organs in pathological process that contributes to the development of multiple organ failure. Thus, according to Pasternak VN, it is

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established that in multiple trauma, liver becomes involved in pathological process due to reduced activity of enzyme systems and the kidneys. The author suggests that these two organs play a great part in organ failure [8, 9]. In the literature Prydruha SM and Gasyuk NV analysed the published data regarding the mechanisms of tissue damage to various organs in different periods of traumatic disease [10]. Kozak DV and Volkov KS noted that damage to organs and tissues serves as morphological substrate of traumatic disease [11]. According to the obtained preliminary results, the development of hypoxemia, caused by chest trauma, affected lipid peroxidation stimulation that caused disruption of cell membranes structure and consequently cell death closure of 'vicious' circle, which in our and other authors opinion cause multiple organ failure with involvement of heart, lungs, liver in the pathological process [12].

The aim of our study was to determine the features of morphological changes in cases of heart, lungs, liver injury after associated chest and both thighs trauma in rats.

Methods

The study was conducted using 36 adult male white nonlinear rats of 200–220 g body weight. Rats were housed under standardized laboratory conditions, with 12 h dark/light cycle and free access to food and tab water ad libitum. All procedures were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and General Ethical Principles of Experiments Using Animals (First National Congress of Bioethics, Kyiv, 2001). The animals were randomly divided into 3 groups: 1 control (C1) and 2 experimental (E1, E2) groups, each comprising 12 animals. The animals in the experimental group underwent thiopental sodium anaesthesia (40 mg/kg intraperitoneal) using a trocar modelled right-closed pneumothorax from fractured ribs and combined them with a broken left and right femur. Skeletal injury was simulated by applying a single shock dosed by a specially designed device on each thigh, which caused a closed fracture [13]. Impact energy was 0.375 J, which corresponded to the injury of moderate severity. Combined injury was simulated by sequential administration of these two injuries. Mortality of animals in each group was E1(12/11), E2(12/9), E3(12/10), E4(12/10), E5(12/9). At the end of the experimental period, the rats were sacrificed by decapitation.

For histological examination the pieces were separated from left ventricle tissues of heart, lungs and liver and fixed in 10% neutral formalin solution, dehydrated and embedded in paraffin blocks [14]. Then the sections, obtained by Sannomiya microtome, were stained with hematoxylin-eosin. Histological preparations were studied using light microscope and SEOSCAN and documented using the camcorder Vision CCD Camera.

Results

Histologically we have found out the beginning of morphological changes in the liver structure of experimental animals in 1 day after the experiment was established. Thus lobular structure still was intacted, sinusoid's gaps were visualized well, and some of them were a little expanded and did not contain any cellular elements. Hepatocytes were increased slightly in size, but most of them saved their organization. Their cytoplasm became enlightened, fine-grained, filled in with vacuoles of various sizes. Some nucleus grew in size, their nuclei were visualized well; the rest of the nucleus were enlightened, pycnotic corrugated (Fig. 1, 2). Area of portal tracts was increased mainly due to the plethora of vessels (Fig. 2). Perivascular oedema and lymph histiocytic infiltration were not observed.

Histological examination of the myocardium of experimental animals in 1 day after the beginning of the experiment proved low-arterial vessels blood supply in epicardium and individual vessels of venous type dilated with mild perivascular oedema. Perivascular stroma was slightly loosened because of oedema. Cardiomyocytes were visualized well; their cytoplasm

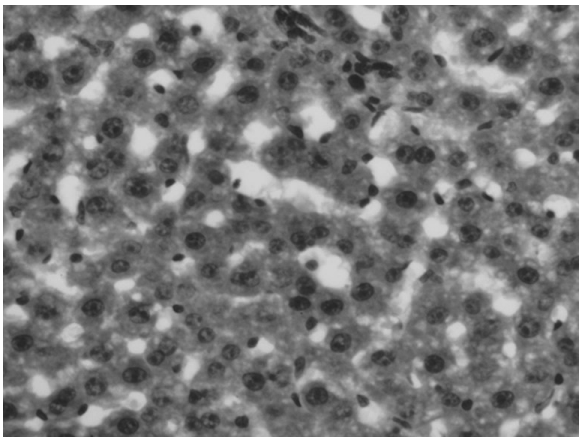

Fig. 1. Liver structure in cases of associated chest and thighs trauma on the 1st day of the experiment. (Significant degenerative changes of hepatocytes, structural changes of nuclei). Stained with hematoxylin and eosin. ×200.

was rich, homogenous; well accumulated pigment. Nuclei were visualized in the main part of the cells. Some nuclei increased in size, karyoplasm was enlightened (Fig. 3). In pericellular spaces we detected a slight swelling, which occurred among the few red blood cells. Some of the cardiomyocytes were fragmented.

Histological examination of lungs of experimental animals revealed that in 1 day tissue blood flow was decreased. Phenomenon of dystonia with spasm of vessel walls was dominated, but land-capillary venous plethora was also observed (Fig. 4). Inter air sac membrane became thick because of a slight oedema, vascular dystonia, and focal perivascular infiltration (Fig. 5). Cellular reaction manifested by leukocytosis and macrophage infiltration. Some of alveoli were expanded; their gaps remained free of fluid. The walls of small bronchus were mainly spasmed, ciliated epithelium surface became flat; in some areas pockets of inflammatory infiltration and exfoliation of the epithelium into the lumen were identified. However, the majority of gaps were free of fluid. The pulmonary pleura remained without any signs of inflammation.

Histological examination of liver of experimental animals in 3 days of the experiment proved the progression of structural changes that resulted in violation of lobular structure and disruption of cells structure. Enlightenment sinusoid was visualized poorly and practically did not contain cellular elements. Beamed organization of cells was violated; cell size was raised significantly by increasing the number of cytoplasmic inclusions. The cytoplasm was mosty enlightened, became fine-grained. The majority of nuclei were hypertrophied, nuclei were well vizualized; some of cores were enlightened, pyknotic corrugated (Fig. 5). Area

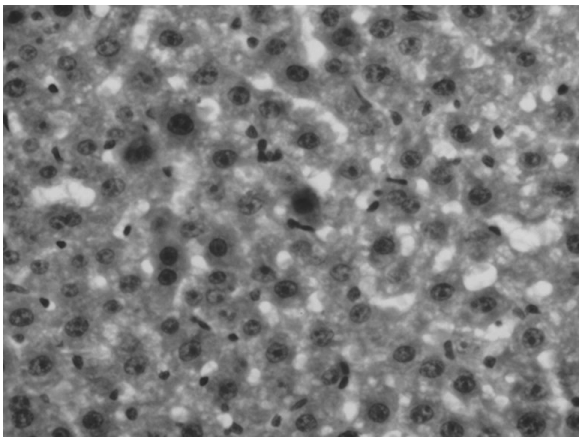

Fig. 2. Liver structure in cases of associated chest and thighs trauma in 1 day after the beginning of the experiment. (Dystrophic changes of hepatocytes, expansion sinusoid). Stained with hematoxylin and eosin. ×200.

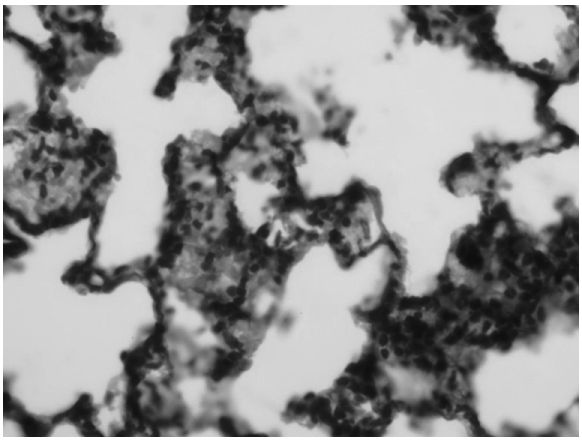

Fig. 4. Lung structure in cases of associated chest and thighs trauma in 1 day after the beginning of the experiment. (Uneven capillary venous hyperemia). Stained with hematoxylin and eosin. ×200.

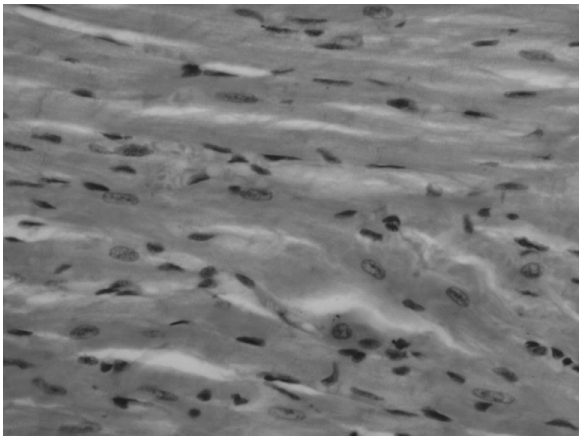

Fig. 3. Myocardium structure in cases of associated chest and thighs trauma in 1 day after the beginning of the experiment. (Slight swelling of pericellular spaces, fragmentation of some cardiomyocytes). Stained with hematoxylin and eosin. ×200.

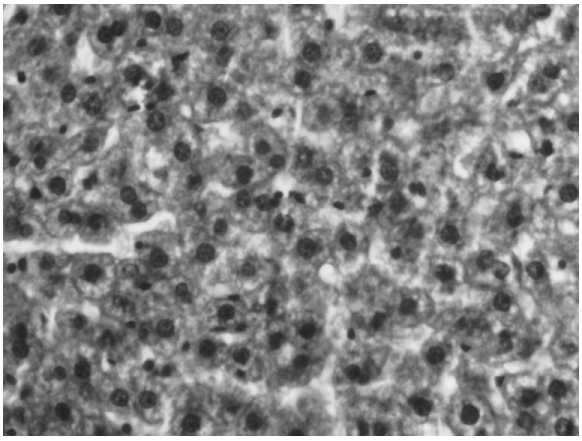

Fig. 5. Liver structure in cases of associated chest and thighs trauma in 3 days after the beginning of the experiment. (Increased dystrophic changes of hepatocytes). Stained with hematoxylin and eosin. ×200.

portal tracts were increased, mainly due to the plethora of lymph vessels and moderate histiocytic infiltration. Perivascular oedema was not observed.

Histological examination of the myocardium in 3 days of the experiment proved the increase of vessels blood supply of both arterial and venous type, their hyperemia and perivascular oedema increased. It leaded to decrease of perivasal stroma and increase of intracellular spaces (Fig. 6). The structure of the majority of cardiomyocytes changed a little. They were located evenly, their sarcoplasm remained homogeneous, cores were partly enlightened, but their contours remained clear. Only a few fibres were replated with colour that indicated of their abbreviation.

In 3 days of the experiment, the thickness of inter air sac membranes because of increasing of oedema, easing elastic and collagen fibres and focal perivascular infiltration, was observed in the lungs of rats (Fig. 7). The cell reaction was manifested by leukocytosis and moderate increase of macrophages, mast cells and lymphocytes. Enlightenment of some alveoles remained slightly enhanced, but the most of them were free of fluid. The walls of small bronchus remained spasmodic, superficial single-row ciliated epithelium became flat. In mucosa the area of focal inflammatory infiltration and exfoliation of the epithelium into the lumen increased. Pulmonary pleura inflammation was not evidenced.

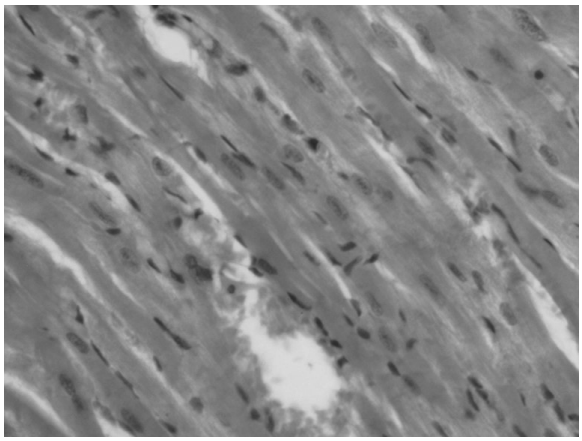

Fig. 6. Structural changes in myocardium caused by associated chest and thighs trauma in 3 days of the experiment. (Decrease of perivasal stroma and increased intracellular spaces). Stained with hematoxylin and eosin. ×200.

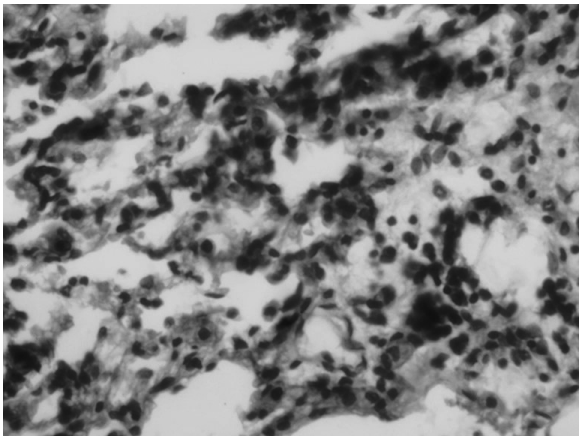

Fig. 7. Lungs structure in cases of associated chest and thighs trauma in 3 days after the beginning of the experiment. (Moderate oedema, vascular dystonia, and focal perivascular infiltration). Stained with hematoxylin and eosin. ×200.

Discussion

It is established that during the first 12 hours since the injury, due to local tissue damage and activation of systemic inflammatory response, acute disorders of vital functions take place [15, 16]. The release of proteases, oxidants, leukotrienes and proinflammatory cytokines under the influence of pathogenic factors causes the damage of lung endothelium that leads to pulmonary oedema. In this study it was proved that thickening of inter air sac membranes in 3 days of the experiment and lesions of alveolar epithelium result in decrease of surfactant production and loss of normal fluid transport that impairs lung function [19, 20]. Results of the study indicate that the associated trauma of chest and both thighs in 1 day after the injury is accompanied by disturbances of hemodynamics in parenchymal organs due to venous congestion that occurs during the development of cardiovascular disease (low-blood supply in arteries of epicardium and single extended venous vessels with a minor perivascular oedema) caused by traumatic shock [17]. In the lungs the system of inflammatory response becomes more active in the form of cellular reactions that are manifested by leukocytosis and macrophage infiltration.

In the most difficult period of maximum likelihood of complications, within 3 days respiratory hypoxia, cardiovascular disorders, metabolic acidosis, and induced programmed cell death may develop [18]. Previously we have found out that associated trauma of chest and both thighs in rats is accompanied by tissue hypoxia in early posttraumatic period and is characterized by the increase in the concentration of lactate and pyruvic acid from the first day of the experiment. We believe that the progressive development of hypoxia and activation of free radical oxidation and potentiate

endogenous intoxication contract artery walls reaction and structural changes on the cellular level.

Conclusions

On the background of increasing pulmonary oedema (thickening of inter air sac membranes) provided by associated trauma of chest and both thighs in rats in only one day of the experiment, hemodynamic changes in heart (low-blood supply of vessels in epicardium and isolated blood vessels of venous type) and liver (mainly plethora of blood vessels) were observed; it leads to morphological changes of myocardium in 3 days, characterized by loosened perivasal stroma, increased intracellular spaces, and structural changes in liver: violation of lobed beam structure and hepatocytes organization, development of degenerative changes.

In the future we plan to investigate the influence of antioxidant correction on morphological changes in lungs, liver and heart after traumatic injuries of chest and hips.

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FREE RADICALS AND INFLAMMATION IN RATS OF DIFFERENT AGE IN CASES OF SODIUM NITRITES AND TOBACCO SMOKE POISONING

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Background. *Due to the wide use of nitrate fertilizers in agriculture and their migration into groundwater and food, the spreading of nitrate poisoning has become epidemic. However, people in the process of life get into bad habits: smoking, alcohol, drugs abuse. All these factors affect health and can cause fatal outcome. In real life, people are often exposed to more toxic factors that lead to general poisoning of the body and damage of many organs.*

Objective. *The research was aimed to study the activity of free radicals and inflammation in rats of different age in cases of sodium nitrite affection with underlying 45-day tobacco intoxication.*

Methods. *The content of nitrite ion (NO_2^-) was evaluated by Gris reaction. The level of pro-inflammatory (interleukin 6 (IL-6) and anti-inflammatory (interleukin 4 (IL-4) cytokines was determined in serum by ELISA method using test kits.*

Results. *It was proved that in rats of different age affected by sodium nitrite with underlying 45-day tobacco smoke intoxication, the content of nitrite ion in serum, liver, lungs and myocardium is increased. After poisoning the animals with the studied toxicants, inflammation was activated in the body that was evidenced by the increased pro-inflammatory cytokine IL-6 and decreased inflammatory cytokine IL-4 in serum.*

Conclusions. *The nitrite ion content in organs was the most significant and inflammation was manifested in the immature rats. In these animals the content of anti-inflammatory cytokines was the lowest.*

KEY WORDS: **tobacco smoke; sodium nitrite; rats of different age; nitrite ions; cytokines.**

BIOMEDICAL SCIENCES

Introduction

The wide spread smoking is a global problem of humanity; many scientists and experts make efforts to solve it. Smoking has a negative impact on the health of the smokers, but also on the health state of people, who do not smoke but are exposed to harmful effects of pollutants entering the atmosphere with tobacco smoke [1]. Numerous scientific studies clearly highlighted the negative influence of smoking on the development of many diseases of different organs and systems of the human body [2, 3].

In recent years, the influence of smoking on free radical processes on the body has been extensively studied. It is established that smoking causes depletion of vitamins C and A, decreases serum levels of other antioxidants, which leads to tissue damage by free radicals [4, 5]. Study of the oxidation-antioxidant status in passive smokers revealed similarity of such changes in active smokers [6].

Due to the increasing technogenic and anthropogenic pollution, the study of combined effects on the body of the most common xenobiotics: heavy metals, nitrates and nitrites, tobacco smoke, medications, is urgent. Considerable attention is attracted to intoxication mechanism of nitrite and nitrate. This happens because of the intensive application of chemicals in the industry, lack of efficient methods for purifying drinking water, high levels of pollution caused by nitrates and nitrites number in foods, especially early fruits and vegetables. Hemic hypoxia, caused by nitrates that penetrate into the body, cause functional and morphological changes in many organs, including kidneys and liver, which can lead to further development of comorbidity [7, 8].

Because of the summation of environmental risk factors, chronic inflammation may develop, which involves all organs and tissues.

Recently, the attention of researchers has been attracted to mediators of immune re-

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sponses – cytokines. Cytokines are produced by all body cells for cell-to-cell interaction and regulation of biochemical processes in cell [9]. Imbalance of cytokines is important for the pathogenesis of toxic affection of the body.

The aim of this research was to study the activity of free radicals and inflammation in rats of different age in cases of sodium nitrite affection with underlying 45-day tobacco intoxication.

Methods

The experiments were conducted on white outbred male rats that were kept on a standard diet at the vivarium of Ternopil State Medical University. The rats were divided into three age groups: the first – immature, weighing 60-80 g, the second – mature, weighing 180-200 g, the third – senile rats, weighing 300-320 g; each age group consisted of two subgroups: an intact control and experimental group. Rats study groups were affected with tobacco smoke for 45 days. The test animals were divided into 3 groups. One of them was administered with sodium nitrite, dose 45 mg/kg 24 hours before the end of the experiment; the second – with sodium nitrite 72 hours before euthanasia. The third group of rats was subjected to toxic affection with tobacco smoke only. Depending on the chronic smoke effect, the model was simulated using a sealed chamber 30 litres in volume, allowing fumigating the animals in free behaviour. Tobacco smoke, formed by combustion of 6 cigarettes "Prima sribna (blue)", containing 0.6 mg of nicotine and 8 mg of tar, was served into it through openings in the chamber. The camera was located 6 animals both within 6 minutes. The animals in the control group were well for 6 minutes in a sealed chamber, but were not subject to smoke.

In 45 days after the beginning of the affection of the animals with tobacco smoke they were taken out of the experiment by euthanasia under thiopental anaesthesia.

Blood, serum, liver, lungs and myocardium of the animals were used for the study. The experimental tissues were used to prepare 10% homogenates using saline.

The content of nitrite ion (NO-2) was determined by Gris reaction [10].

Pro-inflammatory (interleukin 6 (IL-6) and anti-inflammatory (interleukin 4 (IL-4) cytokines levels in blood serum were determined by ELISA methods using test kits. Quantitative assessment of serum concentration in peripheral blood of these cytokines was performed by chemiluminescent enzyme-linked immunosorbent assay using ELISA analyser RT-2100C. Test systems and control serum IL-4, IL-6, Russia, were used according to the test systems protocols. The results of the reaction were determined by a spectrophotometer ULAB-108UA, wavelength 450 nm. These cytokines concentration was evaluated using a calibration curve in picogram per 1 ml (pg/ml) [11].

The research was performed according to the general principles of animal experiments approved by the National Congress on Bioethics (2001, Kyiv, Ukraine) and is consistent with the regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1985) [12]. Statistical analysis of the data was performed using STATISTICA 6.0, parametric Student's t-test and nonparametric Wilcoxon criterion for related samples. Changes considered significant at $p \leq 0.05$ [13].

Results

After rats poisoning with sodium nitrite (SN) during a 45-day tobacco smoke (TS) toxicity we have noticed the increase in content of nitrite ion in serum and organs (Table 1).

In immature rats nitrite ion content in serum increased by 18% till 45 day of TS toxicity. After the poisoning with sodium nitrite (24 hours before the end of the experiment on the 45th day) the content of this indicator increased by 69%, and after the application of toxicants in 72 hours before the study deadline the content of nitrite ion increased by 132%.

A similar increase in nitrite ion content was evidenced in serum of mature and old rats. Using of both toxicants led to increase in its content in 2 times (72 hours after 45-day SN poisoning of TS animals).

In liver of rats of all age, nitrite ion content increased after the affection with tobacco smoke. The use of sodium nitrite as an additional toxicant contributed to the formation of nitrite ion. In liver of the immature rats the rate increased in 3 times, in mature – in 3.5 times. The least sensitive liver was evidenced in the senile animals which nitrite ion content increased in 2.2 times in the final period of the study.

Similar dynamics was observed in lungs of rats of different age. The highest content of nitrite ion was in the groups of rats with underlying 45 day tobacco intoxication which 72 hours before the end of the experiment were administered with sodium nitrite. This rate

Table 1. The content of nitrite ion in serum (nmol/L) and organs (nmol/kg) of rats of different age affected by sodium nitrite with underlying 45 day tobacco smoke toxicity (M±m; n=72)

Research time, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
	blood serum		
intact rats	10.00±0.46	8.20±0.74	8.80±0.56
45 day affection with tobacco smoke	11.80±0.44*	10.20±0.18	10.70±0.46
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	16.90±0.87*	15.30±0.22*	15.80±0.29*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	23.20±0.37*	16.40±0.25*	17.90±0.18*
	Liver		
intact rats	7.60±0.22	3.20±0,16	9.90±0.39
45 day affection with tobacco smoke	10.20±0.61*	5.40±0.51*	11.40±0.43*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	15.60±0.21*	11.00±0.29*	16.10±0.79*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	23.00±0.59*	11.30±0.48*	21.50±0.73*
	Lungs		
intact rats	1.30±0.14	1.00±0.11	1.70±0.19
45 day affection with tobacco smoke	2.90±0.28*	1.60±0.14*	2.60±0.23*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	3.40±0.31*	1.90±0.11*	3.10±0.17*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	4.80±0.21*	3.20±0.13*	4.60±0.32*
	Lungs		
intact rats	2.20±0.14	1.60±0.15	1.80±0.15
45 day affection with tobacco smoke	4.00±0.26*	2.70±0.14*	3.40±0.14*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	4.40±0.21*	2.90±0.17*	3.70±0.20*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	4.90±0.16*	3.20±0.13*	4.10±0.13*

Note: here and in the following tables * – significant changes between intact rats and rats affected with tobacco smoke ($p \leq 0.05$).

increased in the immature rats in 3.7 times, in the mature – in 3.2 times, and in the senile – in 12.7 times compared with the intact control group.

During the study of myocardium of the smoke intoxicated rats almost the same increase in the content of nitrite ion in all age groups (in 1.7-1.9 times higher than normal) was noted. In the rats poisoned with both toxicants this rate also increased: in the immature and old rats it exceeded the intact control in 2.2-2.3 times respectively. In the mature rats the rate increase in myocardium exceeded 2 times.

Thus, the poisoning of rats for 45 days with tobacco smoke caused a moderate increase in nitrite ion content in blood serum and organs of rats of different age. After an additional affection of rats with sodium nitrite, this rate significantly increased ($r \leq 0.05$) and was considerably higher than in the intact animals. This increase in the content of nitrite ion may be caused by activation of free radical processes in the affected organism.

We determined the content of pro- and anti-inflammatory cytokines in blood serum of rats after simultaneous affection with sodium nitrite and tobacco smoke. The results of the study of pro-inflammatory cytokine (IL-6) content in blood of rats of different age groups are presented in Table 2.

In old rats inflammation development was less significant, as evidenced by the increase of IL-6 content in serum after the affection with tobacco smoke in 2.4 times, and after the application of both toxicants in 2.6 times (at the end of the experiment).

Most inflammation was activated in the immature rats. Poisoning of the young animals with tobacco smoke resulted in pro-inflammatory cytokine increase in 3.4 times. In the smoke affected animals, which 72 hours before

Table 2. The content of pro-inflammatory cytokines (IL-6) in serum (ng/L) of rats affected with sodium nitrite with underlying 45-day tobacco smoke toxicity (M±m; n=72)

Research time, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
intact rats	1.91±0.28	3.00±0.30	4.14±0.17
45 day affection with tobacco smoke	6.43±0.21*	8.31±0.19*	9.97±0.29*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	7.74±0.35*	9.35±0.32*	10.94±0.21*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	8.87±0.21*	10.50±0.26*	10.90±0.31*

Note: here and in the following tables * – significant changes between the intact rats and the rats affected with tobacco smoke ($p \leq 0.05$).

the end of the experiment end (45th day of tobacco affection) were administered with sodium nitrite, the content of IL-6 increased in 4.6 times.

Thus, the immature rats were the most sensitive to smoke and both toxins, inflammation in them was developed the fastest and most actively.

The research of anti-inflammatory cytokines (IL-4) content in the studied rats affected with xenobiotics was useful. The results are presented in Figure 1.

The rate of inflammatory cytokines activity is the lowest in the immature rats, in the simul_taneous use of sodium nitrite and smoke the content of IL-4 reaches 45% compared to the norm (on 45th day of smoke intoxication and 72nd hour of sodium nitrite poisoning), as presented in Figure 1.

In the immature and senile rats the content of this indicator after the affection with tobacco smoke was on the same level, 68% of the intact animals' rate. The use of sodium nitrite as an additional toxicant contributed to the disorders in the system of anti-inflammatory cytokines formation. In the old rats after sodium nitrite application, the IL-4 content in tabaco smoke intoxicated rats decreased by 45% and was 55% of norm, in the mature rats in this period it was 59%.

Therefore, sodium nitrite poisoning of rats with underlying tobacco intoxication leads to the imbalance in the system of pro-/anti-inflammatory cytokines, pro-inflammatory indicators are predominant.

Discussion

It is established that after getting into the human body nitrates are restored to nitrites, which lead to the activation of methaemoglobin formation that causes tissue hypoxia. According to literature [7], sodium nitrite in contact with oxyhaemoglobin is a powerful generator of active radicals: HO_2, O_2 OH, NO_2. These metabolites damage biological systems, have a significant cytotoxic action, initiate the process of lipid peroxidation, can reveal a strong oxidizing action interacting with SH-groups of proteins, reduced forms of nucleotides, physiologically active compounds [14].

In the pathogenesis of various diseases, inflammation caused by immune mechanisms is of great importance that is confirmed by the numerous experimental studies [15]. Inflammation is developed in response to damage and penetration into tissues of pathogens with

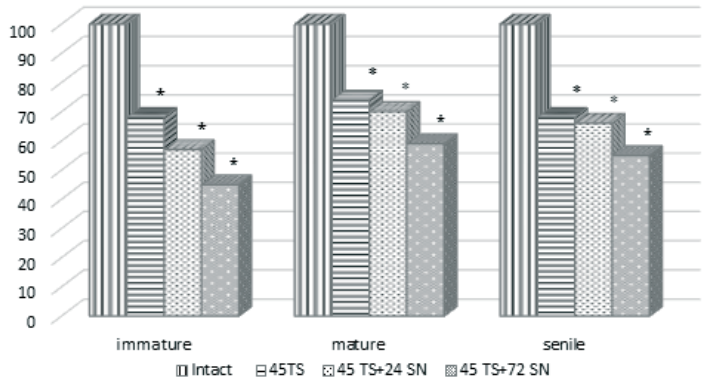

Fig. 1. The content of anti-inflammatory cytokines (IL-4) in blood serum of the rats of different age groups affected by sodium nitrite with underlying 45-day tobacco intoxication, %.

participation of pro-inflammatory cytokines: IL-1, TNF-α, IL-6, IL-8 chemokines. In case of local reactions violation, protective inflammatory response is intensified, synthesis of cytokines is increased; they get into the bloodstream and effect at a system level. In this case pro-inflammatory cytokines affect almost all organs and body systems. [15]

The cytokine imbalance is important for the initiation and progression of inflammation in the body, but the role of cytokines during chronic inflammatory reactions still remains to be determined.

Conclusions

The affection of rats of different age with sodium nitrite with underlying tobacco intoxication leads to nitrite ion accumulation in serum and organs of animals that may be caused by activation of free radical processes in the body. The violation in ratio pro- and anti-inflammatory cytokines was determined. The most significant increase of IL-6 in the immature rats with underlying decrease of anti-inflammatory IL-4 was proved; that is one of the causes of decreased activity of protective systems after injury.

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